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THE PROPERTIES AND DISTRIBUTION OF PAS POSITIVE SUBSTANCES IN THE NERVOUS SYSTEM OF THE SENILE DOG

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According to the observations reported in a study of senile changes in the autonomic ganglion cells of the dog, the cytoplasm of the ganglion cells includes at least three substances that are characterized by giving a positive reaction to the periodic acid-Schiff procedure (6). One of these substances is glycogen which is present not only in the ganglion cells of senile dogs but also in the ganglion cells in dogs of all ages. The remaining two PAS positive substances have been observed only in ganglion cells of dogs 10 years and more of age. Since these deposits occur in the ganglion cells in all dogs in the latter age group that have been examined and have not been observed in the nerve cells of younger animals, i.e., under 10 years of age, it is considered that their deposition is directly related to the process of aging.

One of these substances is present in the cytoplasm of the perikaryon in the form of pigment granules. The remaining PAS positive substance appears in the form of a non-granular cytoplasmic deposition.

The present study was undertaken to determine the distribution of these two PAS positive substances in various parts of the nervous system as well as to determine the nature and properties of these substances by means of histochemical techniques. This is being done with the view that the nerve cells in some portions of the nervous system may lack either one or both of these substances or may contain them in lesser amounts and could thus be considered to be more resistant to age changes of this particular type. This would in turn provide an important tool for further investigation in the aging of nerve cells.

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This investigation was supported by a research grant from the National Institute of Neurological Diseases and Blindness, of the National Institute of Health, Public Health Service.

MATERIAL AND METHODS

The material used in this study includes specimens of brain, spinal cord, autonomic ganglia, and sensory ganglia from 43 dogs ranging in age from 2 weeks to 17 years. The gross age distribution of this series of animals is shown in figure 1. The senile dogs, especially, were obtained from sources in which their case histories could be recorded. All the dogs seemed to be in normal health and were sacrificed because of old age. One dog, a 12 year old Irish Setter, had been blind for a period of two years but had otherwise been in apparent good health.

The tissues were removed immediately after death and were fixed in 10 per cent formalin and in Rossman's fluid. Samples of formalin fixed tissues were set aside for frozen sections; others were dehydrated, embedded in paraffin, and sectioned in the usual manner.

Sudanophilic studies were performed on both frozen and paraffin sections which were stained in a saturated alcoholic solution of Sudan black. Sections were treated for 8 minutes with Sudan black, washed in 70 per cent and 50 per cent alcohol to remove the excess stain, and placed in water from which they were mounted with glycerine. Sections prepared for the demonstration of acid-fast materials were stained with carbolfuchsin overnight at room temperature, then decolorized with 3 per cent hydrochloric acid in 70 per cent alcohol at intervals from 3 minutes to 4 hours. The oxidation potential of the PAS positive materials was studied by means of Lillie's (2) modification of Schmorl's (4) ferric-ferricyanide reduction test whereby deparaffinized sections were treated in a solution

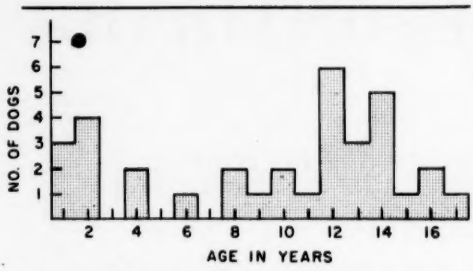

FIG. 1. Gross age of dogs used in this study.

containing 0.75 per cent ferric chloride and 0.1 per cent potassium ferricyanide for 5 to 10 minutes and counterstained with 1:5000 basic fuchsin in 1 per cent acetic acid for 10 minutes. According to this method, sites of reducing substances are blue, and background and nuclei are varying shades of pink.

The periodic acid-Schiff reaction was performed on tissues which had been fixed in 10 per cent formalin and in Rossman's fluid. The positive reaction for glycogen was eliminated by diastase digestion as described by Lillie (1). The periodic-acid Schiff reaction was blocked by acetylation of the sections for 2 to 24 hours at room temperature in a mixture containing 40 parts acetic anhydride and 60 parts pyridine. Deacetylation was accomplished by treatment of acetylated sections with 0.1 N KOH for 40 minutes prior to oxidation with the periodic acid. Sections were also oxidized with peracetic acid and with performic acid prior to treatment with the Schiff reagent in order to demonstrate the presence of the ethylenic linkage in unsaturated lipids such as in ceroids or lipofuchsins. The performic acid and peracetic acid were prepared by the methods described by Lillie (2) and Pearse (3). It was observed that the peracetic acid prepared by Pearse's method reacted similarly to that prepared by Lillie.

Deparaffinized sections were treated with a variety of enzymes prior to treatment with the periodic acid-Schiff reagent. These enzymes included malt diastase, hyaluronidase (Wyeth), pectinase (GBI), lysozyme (Armour), and trypsin (Fairchild). The concentration of the enzyme and duration of digestion is shown in table 1.

Frozen sections of nerve tissue of young and senile dogs which had been fixed in formalin for 8 hours or less were treated with 1 per cent mercuric chloride solution for 10 minutes and then stained by the Schiff reagent for 15 minutes, after which they were washed in 3 changes of bisulfite water and mounted in glycerin jelly in order to demonstrate the presence of plasmalogens. In control sections the treatment with the mercuric chloride solution was omitted.

TABLE 1. EFFECTS OF VARIOUS ENZYME PREPARATIONS ON PAS POSITIVE DEPOSITION IN THE NERVE CELLS OF SENILE DOGS.

Reagent	Concentration	Time of Action	Reactive Group Stainable (+) Unstainable (−)
Malt diastase	1.0 mg./ml. phosphate buffer pH 6.0 + 0.8% NaCl.	1 hr.	+
Hyaluronidase	1 mg./ml. saline, 0.85%	3 to 24 hrs.	+
Pectinase	0.8 mg./ml. acetate buffer, pH 4.2	1 to 48 hrs.	+
Lysozyme	0.5 to 2.0 mg./ml. acetate buffer, pH 6.8	1 to 48 hrs.	+
Trypsin	0.1 mg./ml. phosphate buffer, pH 8.0	15 min.	+
Trypsin	0.1 mg./ml. phosphate buffer, pH 8.0	30 min.	−
Trypsin	0.1 mg./ml. phosphate buffer, pH 8.0	1 hr.	−

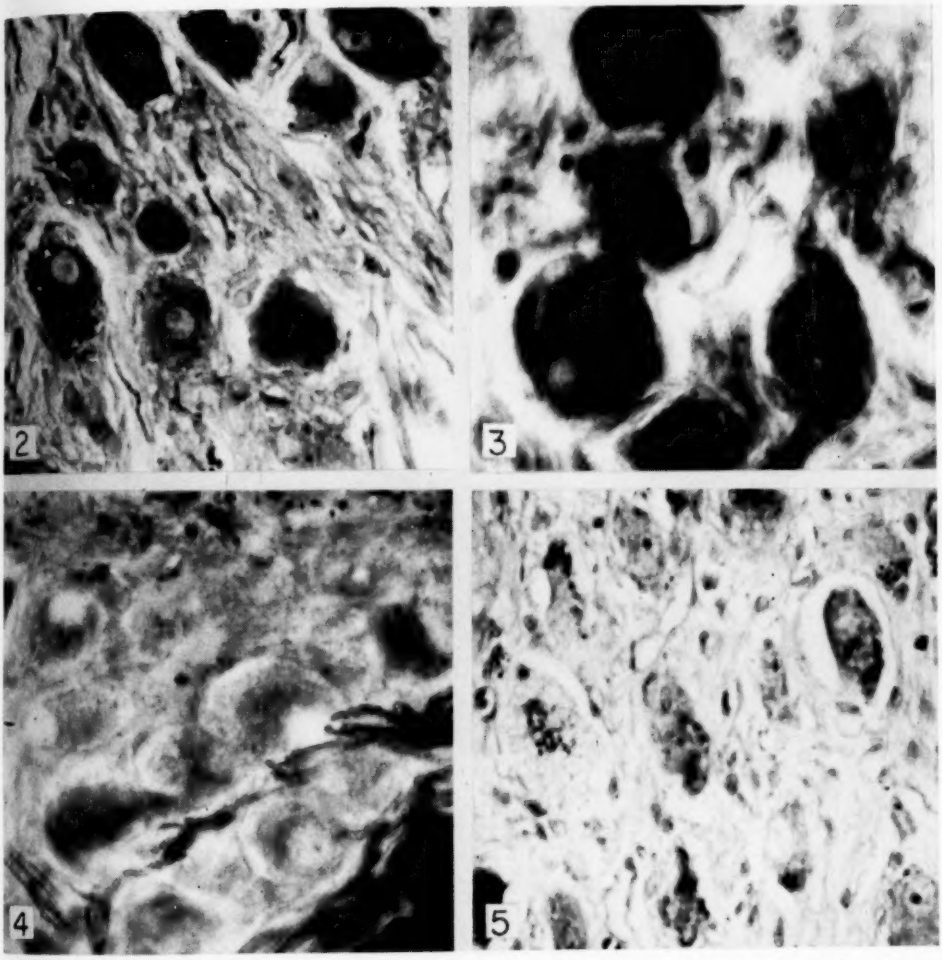

FIG. 2. Photomicrograph of a paraffin section of superior cervical ganglion from 14 year old dog stained with Sudan black, 10X ocular; 44X objective.

FIG. 3. Photomicrograph of a frozen section of a superior cervical ganglion from a 13 year old dog stained with Sudan black, 10X ocular; 44X objective.

FIG. 4. Photomicrograph of a frozen section of an inferior cervical ganglion from a 11 month old dog stained with sudan black, 10X ocular; 44X objective.

FIG. 5. Photomicrograph of a paraffin section of a superior cervical ganglion from a 12 year old dog treated with the carbolfuchsin stain and decolorized with hydrochloric acid. 10X ocular; 44X objective.

OBSERVATIONS

The periodic acid-Schiff positive granules which were observed in the cytoplasm in various parts of the nervous system of all the senile dogs that were examined reacted in a like manner, with one exception to be described later, to all the histochemical tests that were applied. The periodic acid-Schiff positive non-granular deposition in all the dogs which were examined likewise reacted in a similar manner to the various histochemical techniques which were employed. This affords the assumption that the PAS positive granules in all the senile dogs, with one exception, and in all parts of the nervous system have a similar chemical constitution. This assumption is also held for the PAS positive non-granular deposition.

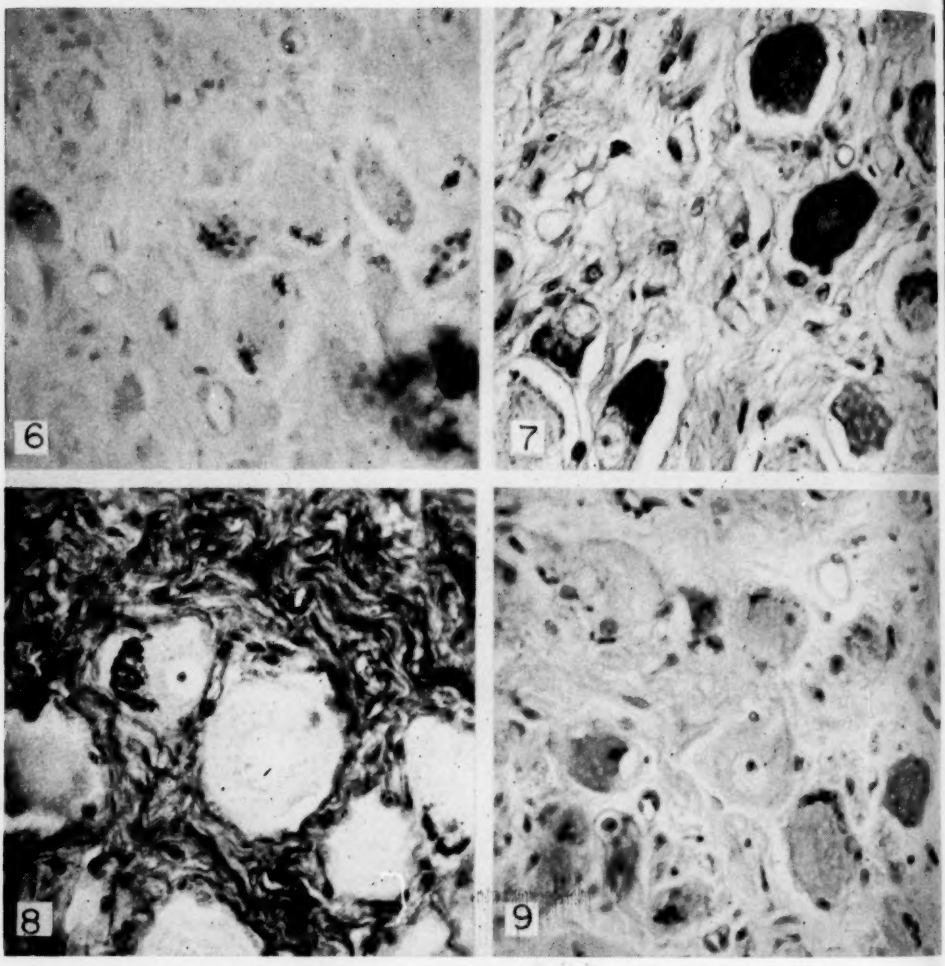

FIG. 6. Photomicrograph of a paraffin section of a thoracic sympathetic trunk ganglion from a 12 year old dog treated with Lillie's modification of Schmorl's ferric-ferricyanide reduction test. 10X ocular; 44X objective.

FIG. 7. Photomicrograph of a paraffin section of a superior cervical ganglion from a 14 year old dog treated with the periodic acid-Schiff reagent. 10X ocular; 44X objective.

FIG. 8. Photomicrograph of a paraffin section of a nodose (sensory) ganglion from a 14 year old dog treated with the periodic acid-Schiff reagent. 10X ocular; 44X objective.

FIG. 9. Photomicrograph of a paraffin section of a superior cervical ganglion from a 14 year old dog oxidized with peracetic acid prior to treatment with the Schiff reagent. 10X ocular; 44X objective.

In paraffin sections stained with Sudan black the PAS positive granules were characterized by a strong sudanophilia whereas the remaining cytoplasm remained unstained (fig. 2). In frozen sections stained with Sudan black a marked sudanophilia was observed in both the granules and in the cytoplasm (fig. 3). It could not be determined by direct observation that the sudanophilic substance was related to the periodic acid-Schiff positive deposition. However, as ordinary lipid solvents, such as alcohol and xylol, do not alter the staining reaction of the PAS reagent on this deposition it may be concluded that the

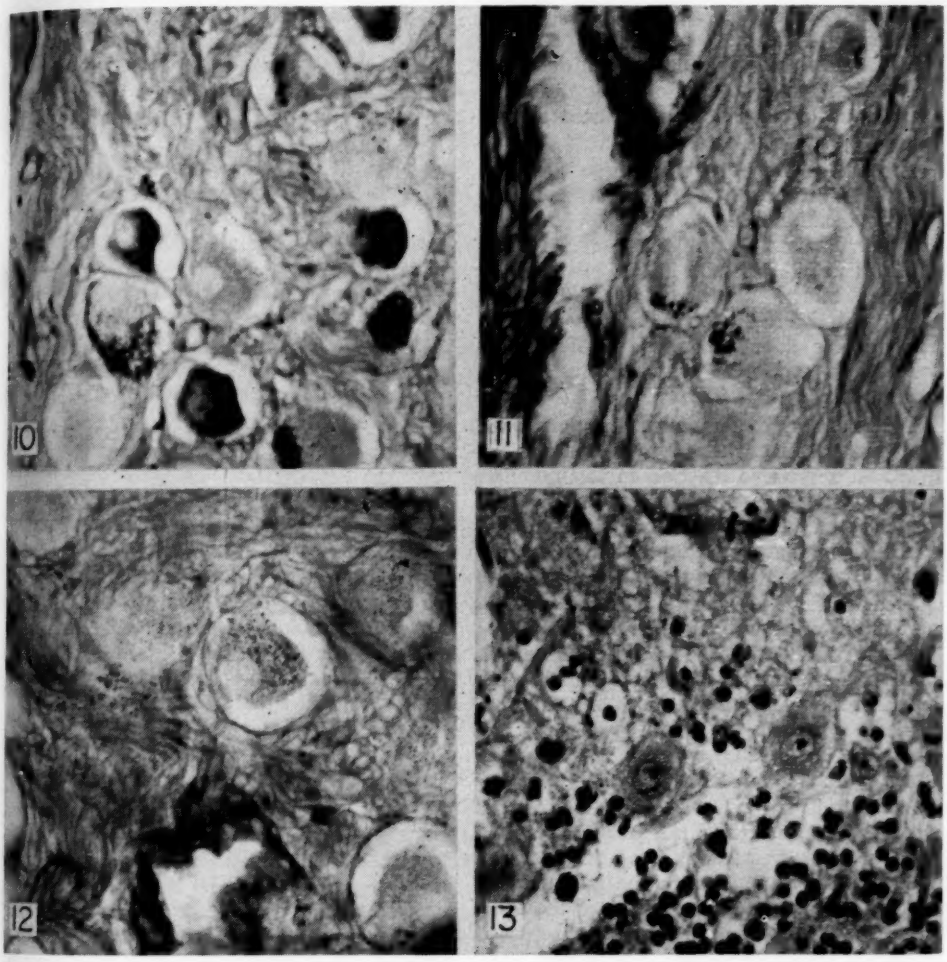

FIG. 10. Photomicrograph of a paraffin section of a superior cervical ganglion from a 15 year old dog which had been incubated in a 0.1. per cent trypsin solution prior to treatment with the periodic acid-Schiff method. 10X ocular; 44X objective.

FIG. 11. Photomicrograph of a corresponding section to that in fig. 14 which had been incubated in 0.1 per cent trypsin for 1 hour prior to treatment with the periodic acid-Schiff method. 10X ocular; 44X objective.

FIG. 12. Photomicrograph of a paraffin section of an inferior cervical ganglion from a 12 year old Irish Setter in which the cytoplasm contained an even distribution of periodic acid-Schiff positive granules which were unlike the pigment granules. 10X ocular; 44X objective.

FIG. 13. Photomicrograph of a paraffin section of the cerebellum of a 12 year old dog stained by the periodic acid-Schiff procedure and showing the Purkinje cells devoid of PAS positive materials and with the adjacent granule cells containing PAS positive granules. 10X ocular; 44X objective.

cytoplasmic sudanophilia of frozen sections is not related to this deposition. In young animals the perikaryon shows very little or no sudanophilia (fig. 4).

Sections of nerve cells of senile dogs stained with carbolfuchsin and treated with hydrochloric acid indicate that the PAS positive granules are characterized by their acid-fast-

ness, even after prolonged exposure (up to four hours) with acid alcohol. The non-granular PAS positive deposition does not show acid-fastness even after being destained as little as three minutes with acid alcohol (fig. 5).

The oxidation potential of these two PAS positive substances was tested with Lillie's (2) modification of Schmorl's (4) ferric chloride-potassium ferricyanide mixture. In sections which were treated in this mixture, the granules stained deep blue and were able to accomplish this reduction in five minutes. The non-granular deposition was unable to accomplish a reduction with the ferric-ferricyanide mixture (fig. 6).

Treatment of deparaffinized sections with the Schiff reagent without prior oxidation resulted in a positive reaction in the granules which was weaker than the reaction of these granules with prior oxidation with periodic acid. The non-granular deposition showed no reaction with the Schiff reagent.

Sections that were treated with the periodic acid-Schiff procedure were characterized by a strong positive reaction in the granules and in the non-granular deposition. In many cells, in which the non-granular deposition was particularly concentrated, the presence of the granules was obscured by this deposition (fig. 7 and 8).

Acetylation of the tissues prior to treatment with the periodic acid-Schiff reagents resulted in a negative, and, in some instances, a slightly positive reaction in the granular elements and a complete inhibition of the reaction in the non-granular elements, whereas if treatment with potassium hydroxide was interposed between the acetylation and oxidation the positive reaction returned.

Oxidation of deparaffinized sections with peracetic acid or with performic acid prior to treatment with the Schiff reagent resulted in a strong positive reaction in the granules which was similar to or only slightly weaker than that resulting from oxidation with periodic acid. No reaction was observed in the non-granular deposition following this procedure (fig. 9).

The observations which have been made from the preceding procedures have been summarized in table 2. The results of these studies present data which indicate that the properties of the PAS positive non-granular deposition differ markedly from the granular elements, the latter possessing the characteristics of a lipofuchsin whereas the non-granular deposition resembles a mucopolysaccharide or mucoprotein. In order to obtain additional data concerning the nature of this non-granular substance the material was treated with a series of mucolytic and proteolytic enzymes.

TABLE 2. SUMMARY OF HISTOCHEMICAL PROPERTIES OF THE PAS POSITIVE PIGMENT GRANULES AND NON-GRANULAR DEPOSITION

Properties or Procedures	Pigment Granules	Non-Granular Deposition
Sudanophilia—frozen sections	+	?
Sudanophilia—paraffin sections	+	–
Acid fast	+	–
Reduction by ferric—ferricyanide	+	–
Schiff positive	+ (weak)	–
Periodic acid Schiff positive	+	+
Acetylation followed by PAS	–	–
Acetylation followed by KOH and PAS	+	+
Performic acid Schiff reagent	+	–
Peracetic acid Schiff reagent	+	–

Deparaffinized sections were incubated at intervals of from 1 to 48 hours in a solution of 0.8 per cent pectinase in acetate buffer at a pH of 4.2. Subsequent treatment of the sections with the periodic acid-Schiff reagent resulted in a strong positive reaction which did not differ from control sections in which the pectinase was omitted from the incubating mixture. In a similar manner, the positive periodic acid-Schiff reaction was not inhibited in sections which were incubated in a mixture containing 0.1 per cent hyaluronidase in 0.85 per cent saline solution for intervals of 3 to 24 hours at 37 C., nor in a mixture containing lysozyme in concentrations of 0.05 per cent to 0.2 per cent buffered at a pH of 6.8.

Deparaffinized sections were incubated with a 0.1 per cent trypsin solution in a phosphate buffer at pH 8.0 for intervals of 15 minutes, 30 minutes, and 60 minutes at 37 C. after which they were subjected to the periodic acid-Schiff procedure. No alteration in the periodic acid-Schiff reactions occurred following digestion in the tryptic mixture for 15 minutes (fig. 10). At the end of 30 minutes of tryptic digestion, the cytoplasm in a majority of the cells did not react in a positive manner to the PAS reaction except for the granular elements, whose staining properties were not in any way altered. The sections which were allowed to remain in the digestion mixture for one hour were characterized by a complete loss of PAS positive material in the cytoplasm of all the neurons except in the granules (fig. 11). The results of these tests have been summarized in table 1.

The nerve cells of one dog in this series, a 12 year old Irish Setter, differed from those in the other senile dogs. Examination of sections of nervous tissue treated with the periodic acid-Schiff procedure revealed that none of the nerve cells contained a PAS positive non-granular deposition but that the cytoplasm in many of the neurons did include PAS positive granules which differed from the granules of the nerve cells of the other senile dogs in their very even distribution in the cytoplasm and by their smaller and more regular size, (fig. 12). Subsequent study of the nervous tissue in this dog revealed that these granules reacted to all the histochemical tests that were employed in a manner identical to the reactions of the non-granular mucoprotein and not to the lipofuchsin granules. Furthermore, those procedures which did not reveal the mucoprotein, such as the performic acid and peracetic acid-Schiff methods and the ferric-ferricyanide reduction test, indicated that lipofuchsin granules were also present in these cells, having been obscured by the presence of large amounts of mucoprotein-containing granules. This is the only instance in which the mucoprotein has been observed in the form of discrete granules.

Although this report deals primarily with the occurrence of periodic acid-Schiff positive substances it is noteworthy that the nerve cells of the senile dogs are characterized by the presence of still another substance which can be demonstrated by the Schiff reagent although it is not periodic acid-Schiff positive. Frozen sections of nervous tissue of young and senile dogs which had been fixed in 10 per cent formalin for 6 to 8 hours were treated with 1 per cent mercuric chloride for 10 minutes prior to treatment with the Schiff reagent. Alternate sections were treated with the Schiff reagent without the previous action of mercuric chloride. Sections of autonomic ganglia of young dogs did not respond to either reaction. Treatment of frozen sections obtained from senile dogs in the aforementioned manner resulted in a mild positive reaction in those instances when the mercuric chloride bath was omitted and in a strong reaction when the sections were acted upon by the mercuric chloride. This is indicative of the presence of "plasmalogens" or acetyl phosphatids in the cytoplasm of the ganglion cells in the senile dog.

The distribution of both pigments and mucoproteins is quite variable throughout the nervous system of the senile dog (table 3) with pigment having a much wider distribution. The occurrence of the mucoprotein seems to be limited to the cytoplasm of neurons of the peripheral nervous system. Its distributions and concentration in autonomic ganglion cells is considerably greater than in sensory ganglion cells. In autonomic ganglia it is present in about 65 per cent of the ganglion cells and in most of these the concentration is such that it obscures the pigment and the rest of the cytoplasm. Its distribution seems similar in all the autonomic ganglia which were studied, including the superior cervical ganglia, the inferior cervical ganglia, the thoracic and lumbar sympathetic chain ganglia, and the coeliac ganglia. In sensory ganglia, on the other hand, it is observed in only 16 per cent of the neurons and it is always present in low concentrations usually at the periphery of the cells.

The distributions of pigment in various portions of the nervous system has been summarized in table 3. The distribution of pigments depends apparently not only on the type of neuron but upon its location. An example of this is to be observed in the granule cell layer of the cerebellum where almost all of the pigmented granule cells are concentrated at the peripheral zone of this layer, adjacent to the molecular layer and in proximity to the Purkinje cells. Very few of the Pur-

TABLE 3. PERCENTAGE OF NEURONS CONTAINING PIGMENTS AND MUCOPROTEINS

	Pigment			Mucoprotein		
	Large	Small	None	Large	Small	None
Autonomic ganglia	23	55	22	54	11	34
Sensory ganglia	10	39	51	0	16	18
Cerebellum Purkinje cells	0	8	92	0	0	100
*Cerebellum granule cells	4	0	96	0	0	100
Cerebellum molecular cells	11	0	89	0	0	100
Cerebellum medullary nuclei	11	44	45	0	0	100
Pontine nuclei	67	26	7	0	0	100
Spinal cord ventral horn cells	64	36	0	0	0	100
Spinal cord dorsal horn cells	27	24	49	0	0	100
Spinal cord intermediolateral column	0	0	100	0	0	100
Cerebral cortex pyramidal cells	68	30	2	0	0	100

*In the Zone adjacent to the Purkinje cells and molecular layer 28 per cent of the granu le cells contain pigment.

kinje cells, although they lie close to a zone of highly pigmented granule cells, contain pigment granules (fig. 13). The data obtained from this survey of the distribution of pigment indicate that efferent neurons have a higher frequency of pigmented cells and a greater concentration of pigments within the cells than do afferent neurons. The exception to this are the neurons of the nucleus myoleioticus in which pigment granules were not observed.

DISCUSSION

In a recent report on the pigments in human autonomic ganglion cells (5) it was concluded on the basis of a variety of histochemical procedures and comparisons with pigments in other organs that the human ganglion cell pigments were lipofuchsin in nature and were closely related to or identical to ceroids. The data obtained in the present study indicate that the periodic acid-Schiff positive granular elements that were observed in the peripheral nervous system and in many parts of the central nervous system in the senile dog were similar in nature to the pigments in the autonomic ganglion cells of man. This conclusion is based on the parallel reactions of these elements to tests for sudanophilia in frozen and paraffin sections, reducability by the ferric-ferricyanide reagent, acid-fastness, ability to stain with the Schiff reagent without prior oxidation, and to the peracetic and performic acid Schiff reagents as well as their insolubility in ordinary fat solvents and their resistance to mucolytic and proteolytic enzymes. An interesting difference may be noted in the occurrence of these similar pigments in the nerve cells of man and dog in that in the latter species they have been observed only in nerve cells of senile dogs whereas in man they have been observed in nerve cells of individuals from 7 to 92 years of age (5, 6).

The observations that resulted from the tests of the non-granular cytoplasmic deposition all indicate that this substance is a mucoprotein. This is based on the fact that this substance is periodic acid-Schiff positive, contains no unsaturated lipids as indicated by its negative reactions to Sudan black and to performic acid and peracetic acid oxidation, and is not metachromatic.

The data obtained from the trypsin digestion tests moreover indicate that the mucopolysaccharide is attached to an insoluble protein and when the latter is digested by the trypsin, the mucopolysaccharide, whose linkage is broken, is washed away during the procedure.

The occurrence of mucoprotein in granular elements in a single aged dog requires further investigation. It is noteworthy that in this particular animal the mucoprotein did not occur as a non-granular deposition in any of the nerve cells. Whether or not this is a race characteristic may be ascertained when other senile dogs of that particular variety became available for investigation. There is furthermore the possibility that the nerve cells in this dog were in a stage of early accumulation of mucoprotein. The availability of large numbers of senile dogs so that various stages of mucoprotein accumulations can be studied, is such, that a study of this nature must be considered as a long term project.

The occurrence of ester phosphatids is not unexpected in the cytoplasm of nerve cells in the senile dog, especially since neutral fats are present in the cytoplasm as demonstrated by Sudan black and since the occurrence of plasmalogens probably represents a stage in the partial oxidation of unsaturated fatty acids with a later stage being represented by lipofuchsin or ceroid granules.

It is apparent from the observations on the distribution of both mucoprotein and lipofuchsin pigment in the nerve cell that different portions of the nervous system and different cell groups within a single portion of the nervous system differ markedly in their degree and frequency in which they contain either one or both of these substances. The distribution of mucoprotein, moreover, is limited to a greater extent than is the distribution of lipofuchsin and has been observed only in the peripheral ganglia. In these ganglia it occurs in greater amounts and with greater frequency in the autonomic ganglia than in the sensory ganglia.

The distribution of lipofuchsin granules is widespread throughout the nervous system of the senile dog but varies considerably in both degree and frequency in different localities. Moreover, the occurrence of lipofuchsin granules seems to be more widespread in efferent neurones than in afferent neurones.

The data presented in this study indicate that various cells or groups of cells in the nervous system of the dog react differently to the process of aging with respect to the accumulation of both mucoprotein and lipofuchsin in their cytoplasm. The mechanism for the differential behavior of these cells during senility cannot be explained at the present time but offers a fruitful device for the study of the phenomenon of aging in the nervous system.

SUMMARY

A study of the nerve cells of 44 dogs, ranging in age from 2 weeks to 17 years, showed that the cytoplasm of these cells that were obtained from dogs that were 10 years of age or more included 3 types of PAS positive substances which differ markedly from each other in their physical characteristics and chemical properties. The histochemical data indicate that one of these substances is glycogen and is found in the nerve cells of dogs of all ages. The other 2 PAS positive materials have not been observed in the nerve cells of dogs below the age of 10 and have been observed in the nerve cells of all dogs over 12 years of age. One of these substances, which is distributed in the form of granules, is a lipofuchsin or ceroid and is identical in its reactions to the nerve cell pigments found in man and in other species. The other PAS positive substance which, with the exception of 1 dog, is present as a non-granular cytoplasmic deposition was shown to have the properties of a mucoprotein. In 1 dog the mucoprotein was present in the cytoplasm of the nerve cells in the form of discrete granules. Both of these substances show a considerable variation in their distribution in different parts of the nervous system. The distribution of the mucoprotein is limited to the nerve cells in the peripheral nervous system and is present in a greater degree in the autonomic ganglion cells than in the sensory ganglion cells. The

lipofuchsin granules have a wider distribution but seem more concentrated in efferent nerve cells than in afferent nerve cells. The chemical nature of these substance is described.

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AGE DIFFERENCES IN VENTILATORY AND GAS EXCHANGE RESPONSES TO GRADED EXERCISE IN MALES

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AGE DIFFERENCES in physiologic systems are more apt to be demonstrated in stress situations. The degree of a response and the speed at which the adjustment takes place may be used as indices of the functional status of the system involved.

Exercise is a stress situation which is both physiologic and readily applied. It affords an opportunity to measure the responses of a number of systems simultaneously.

This report covers the results of experiments designed to answer the following questions:

1. What are the ventilatory and gas exchange responses to exercise in old and young males?

2. What are the effects of work rate and total amount of work done on ventilatory and gas exchange responses in subjects of various ages?

Numerous studies of the effects of frequency of movement and work load (rate of work) have been reported (1, 3, 5, 7, 8, 9, 13, 19, 20, 24). Most of these studies included from 1 to 3 subjects tested at several pedaling (bicycle ergometer) or climbing (stairs) rates and/or several work loads. In general, decrements in efficiency were shown only at movement frequencies of more than 100 or less than 20 per minute. Not even tentative limits are clear for work loads and very little note was taken of the total amount of work done. Hence, a "steady state" was assumed in order to relate work load and mechanical efficiency.

More recent studies (6, 10, 23) have been concerned with the total amount of work done as well as work rate and frequency of movement. Here calculations of efficiency were based on the total or net cost of all the work.

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METHODS

Subjects: Subjects were 19 patients, staff members, and employees of the Baltimore City Hospitals. Five subjects were 25 to 29 years old, 6 were 58 to 70 years old, and 8 were 74 to 85 years old. A potential subject was excluded from the experiment only if he was 1) non-ambulatory, 2) unable to perform the work, or 3) considered by his physician to be a poor clinical risk in this experimental situation.

Experimental Procedure: On the morning of the experiment subjects were brought to the testing room in a basal state, placed on the exercise bed, and fitted with ECG electrodes on both legs and the chest at the precordial V-5 position. After resting for thirty minutes the subject was fitted with a face mask connected through mercury valves to outdoor air and to the collection spirometers. Two 40 L. samples of expired gas were collected for estimation of basal metabolic rate.

The subject then performed a predetermined work bout by cranking an electrodynamic brake bicycle ergometer manually. After exercise, the subject rested quietly for a forty minute recovery period.

Instrumentation: A pair of alternating 10 L. collection spirometers was arranged so that during and after exercise the subject's expired air was collected in serial 10 L. blocks until 200 L. of gas had been expired and then in 40 L. blocks until the end of the forty minute recovery period. A 50 cc. portion of each block of gas was collected over mercury for Haldane analysis of oxygen and of carbon dioxide concentration (18).

The ergometer used in these experiments was similar in design to others which have been described in detail (11, 14, 22). Unlike some of these, however, it was designed for arm exercise at work rates between 40 and 1500 Kg.M./min. and cranking rates between

20 and 120 revolutions per minute. The ergometer was calibrated by standard engineering practice so that the output voltage could be converted to work rate values (kilogrammeters per minute). During work the output was recorded continuously so that the work rate at any instant was available and the total work for all or any part of the exercise period could be estimated. Heart rate, respiratory rate, a one minute interval mark, and the time required to breathe each 10 L. of gas were recorded continuously during the experiment. Time was estimated to the nearest one hundredth of a minute. Zero time was the beginning of exercise.

Calculations: All gas volumes were reduced to standard conditions (0° C., dry and 760 mm. Hg) (2). Barometric pressures were obtained from a mercury barometer read in the laboratory at the time of the experiment.

Ventilation volumes were obtained by dividing the reduced volume of each 10 L. spirometer by the time required to fill it. Carbon dioxide elimination was calculated as the product of the ventilation volume and the difference of concentration of carbon dioxide between the expired air and the inspired air. The oxygen uptake values were similarly determined except the inspired concentration was corrected for differences in inspired and expired gas volumes according to standard BMR procedures (18).

The excess oxygen uptake, carbon dioxide elimination, or ventilation volume required for a given work bout was calculated as the amount in excess of the extrapolated asymptote of the recovery curve (fig. 1).

The asymptote was determined by averaging the rate of ventilation, carbon dioxide elimination, or oxygen uptake for the blocks

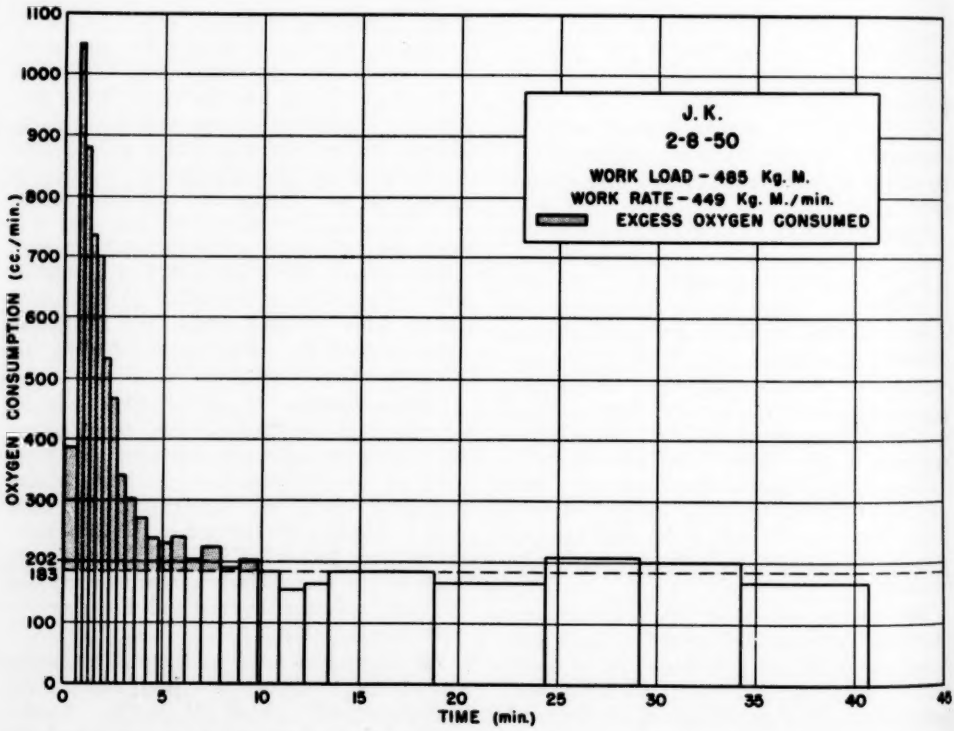

Fig. 1. Oxygen uptake (cc./min.) is plotted against time (minutes) for a single experimental run on J. K., aged 68. The broken line represents the asymptote of the recovery curve (183 cc./min.). The basal oxygen consumption was 202 cc./min. The stippled area above the asymptote represents the total excess oxygen consumed from the beginning of exercise (zero time).

of gas occurring after these rates had fallen to near (usually below) resting levels. Although an attempt was made to determine the point at which the faster components of recovery were complete and to use all blocks of gas thereafter to compute the asymptote for a given estimate, it made little difference (on the average less than 5 per cent of the estimate of the asymptote) whether the point of change in slope or the last ten minutes of the forty minute recovery period was used. For practical purposes, then, averages were taken on an increasing sum of all samples starting with the end recovery sample. When a consistant increment of rates in excess of the average was noted, the point of change in slope was taken as the asymptote for each individual curve.

Net efficiencies were calculated as the caloric value of the work measured by the ergometer (1 calorie = 426.4 Kg.M.) divided by the caloric value of the excess oxygen consumed during and after that work (1 L. of oxygen at an R.Q. of 1.0 = 5.047 calories).

The t-test (21) was used to estimate the level of significance between various pairs of means. Differences were considered significant if the probability of their occurrence by chance was less than 0.05.

Experimental Design: Each subject performed 4 different total amounts of work at a fixed rate and a fixed total amount at 3 different rates. Table 1 gives the average rate or total amount for each group. The fourth total amount and the third work rate represent the same work bout. In addition, 6 of the subjects in the 74 to 85 year group performed 206.0 ± 4.17 Kg.M. of work at 141.7 ± 1.28 Kg.M./min. Nominal cranking rates were 48, 61, 73, and 87 revolutions per minute.

Prior to the day of his initial test each of the older subjects was familiarized with the face mask and the operation of the ergometer. Although the subject's ability to work at a rate of 450 Kg.M./min. was determined during this trial run prolonged effort at such high rates was avoided. Only one of these older subjects had more than one practice session.

The young subjects did not have trial runs except for one of the 5 who had cranked the ergometer many times prior to the initial test in this series.

RESULTS

Maximum Responses: Figure 2 shows, for each age group, the average maximum responses measured for ventilation volume, carbon dioxide elimination, and oxygen uptake

TABLE 1. AVERAGE TOTAL AMOUNTS OF WORK PERFORMED BY EACH GROUP OF SUBJECTS AT AN AVERAGE RATE OF 442.0 ± 2.15 KG.M./MIN.

Age Group	N	Total Work (Kg.M.)						
25-29 Yr.	5	132.5 ± 3.10(4)	203.0 ± 5.38	346.6 ± 4.66	497.2 ± 4.72	609.4 ± 4.01	866.8 ± 6.69	1036.7 ± 35.17(3)
58-70 Yr.	6	134.6 ± 3.53(5)	220.0 ± 10.86	351.5 ± 8.04	499.7 ± 5.85	584(2)		
74-85 Yr.	8	133.1 ± 4.20	219.3 ± 5.15	332.6 ± 8.06	487.5 ± 16.40(6)			

AVERAGE WORK RATES AT WHICH EACH GROUP OF SUBJECTS PERFORMED AN AVERAGE TOTAL AMOUNT OF 497.4 ± 3.13 KG.M.

Age Group	N	Work Rate (Kg.M./Min.)						
25-29 Yr.	5	137.0 ± 2.10	264.0 ± 5.80	450.0 ± 4.36	576.1 ± 10.07	809(2)	1068(2)	
58-70 Yr.	6	139.0 ± 3.19	259.8 ± 5.54	442.7 ± 6.63	575.3 ± 5.44(4)			
74-85 Yr.	8	135.0 ± 1.43	248.0 ± 5.38	*412.8 ± 4.44(6)				

(2) – (6) = Number of subjects where N is less than normal for an age group.
*Rate significantly less than similar rates for other age groups with P <0.001.

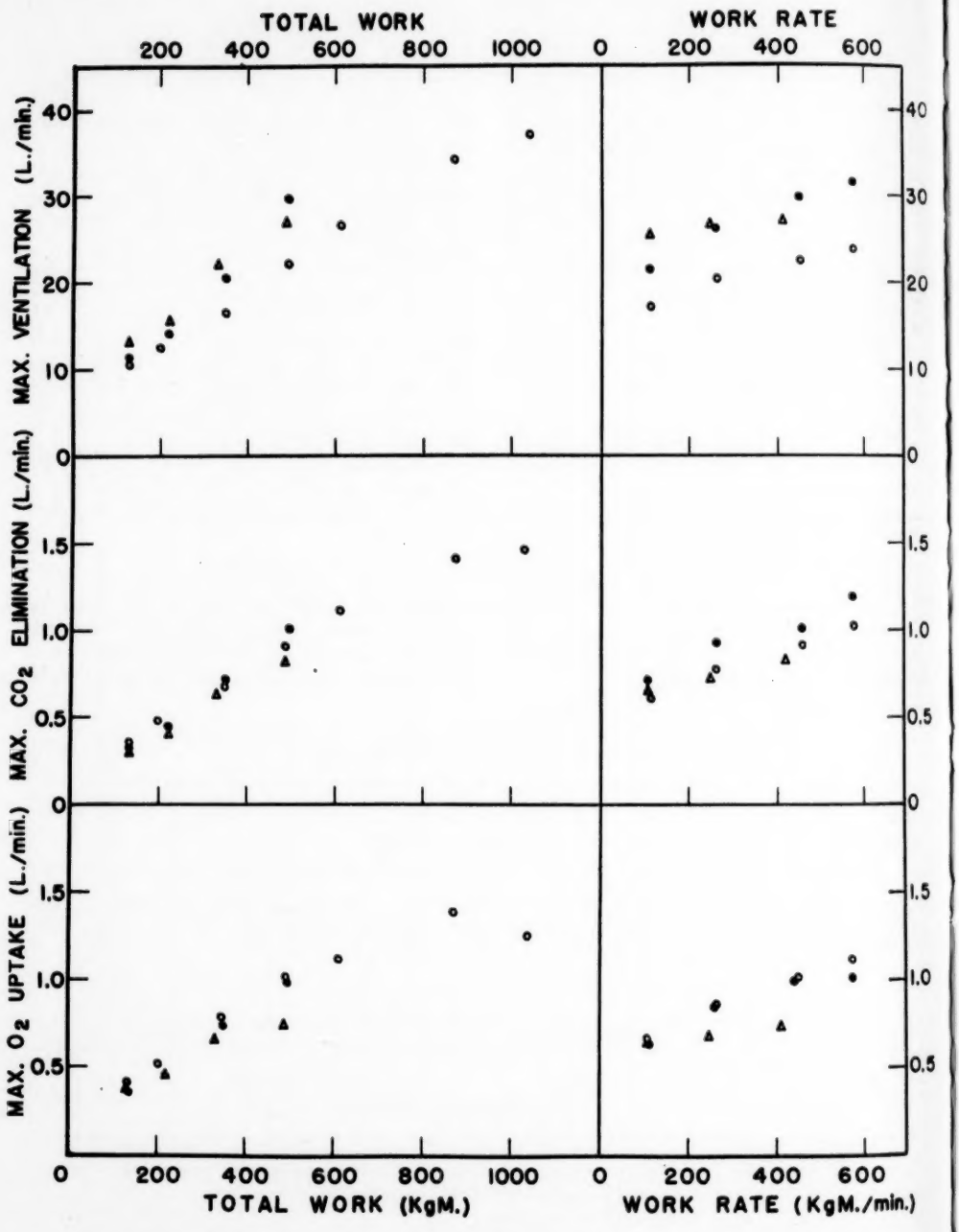

Fig. 2. Maximum oxygen uptake, carbon dioxide elimination, and ventilation volume are plotted against total work and work rate for three age groups: 25-29 yr., O; 58-70 yr., ●; and 74-85 yr., △.

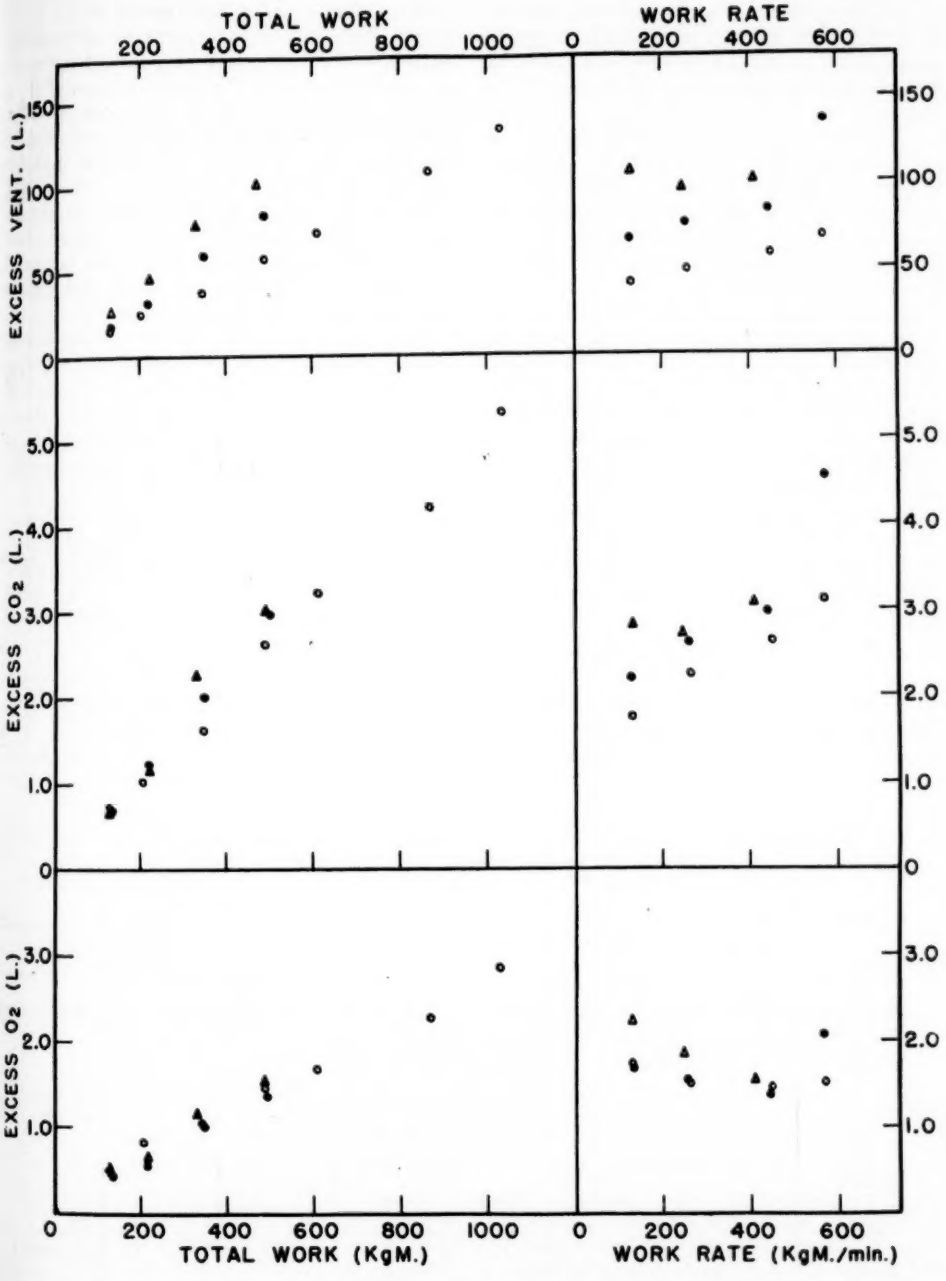

Fig. 3. Total excess oxygen uptake, carbon dioxide elimination, and ventilation volume are plotted against total work and work rate for three age groups: 25-29 yr., O; 58-70 yr., ●; and 74-85 yr., △.

plotted against total amount of work done and work rate. Both groups of older subjects showed higher average ventilatory responses than did the young group at all levels of work, while maximum carbon dioxide elimination responses were similar for all groups of subjects at all levels of work. Moreover, only the oldest subjects showed impaired maximum oxygen uptake at the two highest rates of work they performed. Maximum oxygen uptakes were similar at these levels for the younger subjects and similar for all subjects at other levels.

Significant differences were found only for 1) maximum ventilation volume between the young subjects and the two groups of older subjects at all work rates and 2) maximum oxygen uptake between the oldest subjects and the two groups of younger subjects at work rates of 267Kg.M./min. and 450 Kg.M./min. (500 Kg.M.).

Maximum responses of each of the three measures increased more with increase in total work done than with increase in work rate. This difference was more pronounced in the oldest group of subjects.

Total Excess Responses: Figure 3 shows for each age group, the average total excess ventilation volume, carbon dioxide elimination, and oxygen uptake plotted against total amount of work done and work rate. Each group showed a greater excess ventilation volume than any younger group at all work levels. Both total excess carbon dioxide elimination and total excess oxygen uptake were greater for the oldest subjects measured than for the younger, only at the highest and lowest rates of work (135 Kg.M./min. for the oldest age group and 586 Kg.M./min. for the middle-age group).

Of these differences in total excess responses, only the differences in ventilatory response between the two younger groups at the two lowest total amounts of work, 135 Kg.M. and 202 Kg.M., fail to be significant.

The effect of increasing total work was greater than the effect of increasing work rate on the excess ventilation volume, carbon dioxide elimination, and oxygen uptake. However, all of the responses of the oldest subjects measured were accentuated at work rates of 135 Kg.M./min. (for the oldest age group) and 586 Kg.M./min. (for the middle-age group) as indicated above. In general, significant increases in excess carbon dioxide elimination in older subjects occurred at the same work levels as significantly increased excess oxygen uptakes.

Net Efficiency: Table 2 shows, for each age group, the average net efficiency for each total amount of work and work rate. The oldest subjects showed reduced efficiencies at

TABLE 2. AVERAGE NET MECHANICAL EFFICIENCY (PER CENT)

Age Group	Total Work (Kg.M.)						
	135	202	350	494	597	853	997
25–29 Yr.	13.42 ± 0.69	12.10 ± 1.31	15.11 ± 0.18	15.95 ± 1.01	16.74 ± 0.44	17.95 ± 0.35	16.68 ± 0.28
58–70 Yr.	14.59 ± 1.38	17.87 ± 1.53	15.64 ± 0.72	17.39 ± 1.74			
74–85 Yr.	13.55 ± 0.92	16.02 ± 1.32	14.32 ± 1.68	14.99 ± 1.11			
Age Group	**Work Rate (Kg.M./min.)**						
	135	267	449	586			
25–29 Yr.	13.56 ± 0.50	15.24 ± 0.79	15.95 ± 1.01	15.98 ± 0.71			
58–70 Yr.	14.35 ± 0.61	15.08 ± 0.88	17.39 ± 1.74	10.66 ± 0.76			
74–85 Yr	11.04 ± 0.96	12.27 ± 0.89	14.99 ± 1.11				

the two lowest rates of work they performed (135 Kg.M./min. and 267 Kg.M./min.), while the middle-age group showed lower efficiency than the young group at the highest rate of work they performed (586 Kg.M./min.). In addition, the young subjects were less efficient than the older groups at the total amount of work of 202 Kg.M.

When the oldest group performed the lowest rate of work (135 Kg.M./min.) at another total amount of work (200 Kg.M.) the efficiency of their performance was 11.18 ± 0.85 per cent as compared to 16.02 ± 1.32 per cent for a high rate (450 Kg.M./min.) at the same total amount (200 Kg.M.).

All of these differences in efficiency were statistically significant.

Time of Maximum Response: Table 3 shows an average for all subjects* of the midpoint time of the metabolic period measuring the maximum response of ventilation volume, carbon dioxide elimination, and oxygen uptake. At the low total amounts of work the response time estimate is limited on the low side by initial measurement period duration, which is determined by the ventilatory response to the exercise stimulus (442 Kg.M./min. rate of work for all total amounts). The maximum response may be further fixed around the second 10 L. measurement period by contamination of the early part of the first measurement period with various lag components, both physiologic and instrumental.

At the low rates of work, however, maximum responses occurred before the end of exercise. At the lowest rate of work (135 Kg.M./min.) a plateau, defined as consecutive periods within 5 per cent of the maximum response, was attained in 12 of 19 subjects. There was no age difference in the distribution of these plateaus. At all of the higher work levels, maximum responses occurred at or shortly after the end of exercise. On the average, maximum oxygen uptake occurs earliest, followed by ventilation volume and carbon dioxide elimination.

*There were no significant age differences in time of maximum response.

TABLE 3. AVERAGE TIME FOR ALL SUBJECTS OF MAXIMUM RESPONSE OF VENTILATION VOLUME, CO_2 ELIMINATION, AND O_2 UPTAKE FOR VARIOUS WORK LEVELS

Response	Time (min.) of Maximum Response for Work Rate of 442 Kg.M./min. and Nominal Total Amount and Duration of:						
	135 Kg.M. (0.30 min.)	202 Kg.M. (0.45 min.)	350 Kg.M. (0.78 min.)	494 Kg.M. (1.10 min.)	597 Kg.M. (1.33 min.)	853 Kg.M. (1.90 min.)	997 Kg.M. (2.22 min.)
Ventilation Volume	1.30	1.34	1.29	1.33	1.42	1.86	2.28
CO_2 Elimination	1.32	1.48	1.37	1.54	1.56	2.01	2.39
O_2 Uptake	1.24	1.22	1.11	1.21	1.35	1.90	2.12

Response	Time (min.) of Maximum Response for Total Amount of Work of 497 Kg.M. and Nominal Rate and Duration of:				
	135 Kg.M./min. (3.70 min.)	267 Kg.M./min. (1.88 min.)	449 Kg.M./min. (1.10 min.)	586 Kg.M./min. (0.92 min.)	
Ventilation Volume	3.05	1.80	1.33	1.19	
CO_2 Elimination	3.04	2.06	1.54	1.36	
O_2 Uptake	2.52	1.67	1.21	1.05	

DISCUSSION

The variations in response of ventilation volume, CO_2 elimination, and oxygen uptake to different levels of exercise make it clear that both total amount of work done and work rate must be controlled if exercise is to be used as a standardized stress situation. This is equally true for estimates of either maximum responses or total excess responses.

During and after exercise rapid changes in ventilation volume, carbon dioxide elimination, and oxygen uptake based on the expired air could not be measured continuously. Instead, a large number of small volumes were used for these estimates. The use of a constant 10 L. volume of expired gas permits greater time differentiation at higher ventilation volumes (1.00 minute periods at 10 L. per minute, 0.50 minute periods at 20 L. per minute, and 0.33 minute periods at 30 L. per minute). Thus, peak responses at high maximum ventilation volumes were determined more frequently than those falling on a curve of low ventilation volumes.

The variation in the temporal distribution of the maximum values for oxygen uptake, carbon dioxide elimination, and ventilation volume is consistent, if not significant. The early rise of oxygen uptake reflects the demand for and utilization of oxygen by the exercising muscles and the freedom of the transport system from control or modification other than limitations of the system itself. On the other hand, ventilation and the carbon dioxide content of the blood exert modifying influences on one another. With increasing ventilation, the maximum carbon dioxide elimination will lag behind maximum ventilation volume by at least the amount of time required for the lungs to be cleared of the carbon dioxide removed from the blood. This is not necessarily true, however, when the metabolic contribution of carbon dioxide to the blood has leveled off in the face of increasing ventilation. This might result in a tendency toward a net decrease in the carbon dioxide content of the blood. In this situation, a subsequent leveling off of ventilation volume at a suitably short interval (about 0.5 minutes) might result in simultaneous maxima for ventilation volume and carbon dioxide elimination.

We are concerned here with two types of exercise. The first is done at a high rate of work so that the exercise is completed before the responses have reached their maxima which therefore will occur at or after the end of exercise.

In the second type of exercise encountered (500 Kg.M. at 135 Kg.M./min.), the maxima occur before the end of exercise. In 12 of the 19 subjects values for all measures remained within 5 per cent of maximum until the end of exercise. No systematic trend was noticed in any of the responses after the maximum was reached.

The inability of the older subjects to work at levels higher than those indicated in table 1 may be associated with their inability to maintain adequate oxygen uptake at these work levels (fig. 2). None of the older subjects admitted tiredness of the arms, while most of them complained of difficulty in "getting their breath."

Although the older subjects have generally greater maximum responses of ventilation volume than do the young, examination of the curves of maximum response plotted against work rate and total work suggest that this trend does not hold for the oldest subjects at the highest levels of work. While the middle group of subjects actually increases maximum response faster with increase in work level than does the young group, the oldest group shows the same (at increasing total work) or lower (at increasing work rate) rate of increase than does the young group. Both the increased response and subsequently diminished response might be explained in a variety of ways. The increased response may be the result of decreased efficiency of removal of gases from the gas and blood system of the lungs and thus be dependent on such things as: 1) total pulmonary blood flow, impairment or obliteration of functional capillary units, and/or shunting of flow past large groups of such units on the blood side, 2) decreased ventilation of alveolar spaces because of collection of water, uncoordinated constriction of bronchiolar-alveolar passages, gross anatomic changes and/or increased effective dead space on the gas side, and/or 3) changes in the diffusion or transport systems at the blood-gas interface. On the other hand, the decrease in response, seen most clearly in the oldest subjects, may be the result of changes in the size, shape, and mobility of the chest cage stemming from such things

as rigidity of the bony structure, deterioration of the intercostal or abdominal muscles, and/or relative enlargement of the heart or other internal organs or spaces impinging on the diaphragm directly or indirectly. Moreover, any combination of the above factors might be used to describe the responses of a specific individual.

Since the ventilatory responses to exercise were always higher in the older subjects than in the young subjects, it is not necessary to consider the possible effects of mechanical limitation of ventilation in this discussion. One might consider, however, the failure of the oldest subjects to show a greater ventilatory response than the middle-age group at the highest levels of work (fig. 2). It is possible that an inadequate ventilatory reserve (ventilatory capacity—ventilatory response) in the oldest subjects may be responsible for these smaller increases in ventilatory response with increase in exercise level.

Although the two older groups of subjects had similar maximum ventilation volumes, the oldest group had greater total excess responses than did the middle-age group. Thus, the oldest subjects must have continued to ventilate excessively for a longer time than the middle-age group.

While the ventilatory responses supported maximum carbon dioxide elimination in the old men at levels found in the young subjects, maximum oxygen uptakes were significantly less in the oldest subjects than in the young at the highest levels of work. These impairments of oxygen uptake may be associated with the oldest subjects' inability to work at higher work levels. The maintainance of oxygen uptake may be dependent, secondarily, on a non-ventilatory system, such as pulmonary perfusion, blood flow, or diffusing capacity, which showed limited response in the oldest subjects at the highest levels of work.

Cohn and associates (4) have reported standards for the maximal diffusing capacity of the lungs of normal male subjects. They state that, on the average, the diffusing capacity seems to decrease with age at the rate of approximately 10 units of diffusing capacity every 18 years. They point out that their estimates of diffusing capacity are influenced by the permeability for oxygen of the pulmonary membrane and its area, a function of the number of alveolar capillaries involved in the gas exchange. A reduction of the functional capillary bed may be important indeed in view of the findings of Kirk and Laursen (12) which indicate that the permeability of preparations of the intima and media of human aorta for oxygen, as well as carbon dioxide, nitrogen, and some non-gaseous solutes, tends to increase with age.

Excess oxygen uptakes were significantly higher in the older subjects than in the young subjects at the highest and lowest rates of work they performed. These differences can be considered as differences in mechanical efficiency if one merely wishes to compare the energy equivalent of the external work performed and the energy equivalent of the excess oxygen required to do that work. This, of course, does not imply any assessment of muscle energetics.

Differences in the effects of cranking rate, muscle loading, or coordination ability between old and young subjects may have caused the differences in efficiency found in the present data.

The range of cranking rates used in the present study was between 48 and 87 R.P.M. This is well within the range of uniform effect of cranking rate (20 to 100 R.P.M.) found by previous investigators (5, 7, 13) for young subjects.

The effect of muscle loading might serve as an explanation of the decreased efficiencies at the high rate of work if one were to postulate a decrease in efficiency at or near maximum load. This would certainly not apply to the moderately loaded muscles at the low rates of work, however.

Moreover, the older subjects were less efficient than the young at those rates of work where good coordination of muscular effort was necessary to maintain either a high level of output or a uniform output. A possible cause of poor coordination is derangement in the timing of contraction and relaxation of antagonistic muscles. In such a situation the antagonists could be working against themselves for only a small part of each contraction-relaxation cycle while the sum of useless work over many such cycles might be appreciable. The decrease with aging in conduction velocity of human ulnar nerves which is not associated with any difference in arm length (16) and the even larger decrements

in pursuit and digital reaction speed between the ages of 30 and 80 years (15) suggest a possible basis for the argument for coordination failure.

The reduced efficiency values for the youngest subjects occurred at that total amount of work (202 Kg.M.) which 4 of these 5 subjects were asked to perform without having operated the ergometer previously (without practice). We were unable to demonstrate this effect in 14 subjects between 25 and 50 years of age. They showed no difference between two performances of a work level of 500 Kg.M. at 135 Kg.M./min. even when as many as 5 practice bouts were held on separate days between the two tests. The difference in these findings is probably best explained by the difference in duration of the work bouts, 27 seconds for former (202 Kg.M.) and 3 minutes 43 seconds for the latter. This would seem to suggest that all the practice required by the young subject is accomplished during the first few seconds of exercise.

SUMMARY

A group of five young men (25 to 29 years old) and 2 groups of older men (6, 58-70 years old and 8, 74 to 85 years old) performed 4 different total amounts of work (135-500 Kg.M.) at a fixed work rate (450 Kg.M./min.) and a fixed total amount of work (500 Kg.M.) at 3 different work rates (135-450 Kg.M./min.) on a manual bicycle ergometer operated when the subject was recumbent. Ventilation volume, carbon dioxide elimination, and oxygen uptake were estimated for small blocks of expired gas (10 L. and 40 L.) during exercise, a pre-exercise basal period, and a forty minute recovery period.

Estimates of the maximum responses attained indicated that:

1. Older subjects attained higher levels of ventilation volume than did the young.

2. At the higher levels of work the older subjects showed an impairment of oxygen uptake which was not associated with any impairment of carbon dioxide elimination.

Estimates of the total excess responses indicated that:

1. Older subjects ventilate more air per unit of work done or oxygen utilized than do the young.

2. At high and low rates of work the older subjects require more oxygen for a given amount of work than do the young.

It was suggested that the observed inability of the older subjects to work at higher levels of work than those indicated above was associated with the impaired oxygen uptake.

The reduced mechanical efficiencies found in the older subjects were attributed primarily to the failure of the older man to coordinate his movements as well as the young.

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THE INFLUENCE OF AGE ON PHOSPHATASE ACTIVITY IN THE LIVER OF THE MOUSE

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THE AGING of the mouse liver has as yet been little studied. Andrew, Brown, and Johnson (1) investigated the process histologically and reported that senility is associated with changes in both the nuclei and the cytoplasm of the parenchymal cells. Kochakian and Fox (14), as a part of a study of the effects of castration on alkaline and acid phosphatases of the kidney, liver, and intestine of the male mouse, noted that the organs of normal 155 day old mice contained greater amounts of alkaline phosphatase than those of 75 day old animals. The acid phosphatase activity, on the other hand, was lower in the older animals. These reports suggested the desirability of further study.

Alkaline and acid phosphatase activity can be studied qualitatively through use of the histochemical method of Gomori (11) and quantitatively by biochemical methods (20). Study of a tissue under the same conditions of experiment by qualitative and quantitative methods is of great interest in that it provides the opportunity of searching for a morphologic basis for any observed functional changes. A preliminary report of a histochemical and biochemical study of the alkaline and acid phosphatase activity of the mouse liver as a function of age has been published elsewhere (33). Since that report, however, several papers have appeared discussing factors which influence the reliability of the histochemical methods (16, 21, 23, 24, 32). At the present moment there is lack of agreement as to the validity of the method when applied to mammalian liver, although the liver of the mouse has not been specifically investigated. Despite great care in the preparation of the histochemical material with respect to fixation and length of incubation, it was decided to withhold the final results obtained therefrom until such time as it is certain that they are not based on a false premise. Thus only the quantitative chemical data on mouse liver phosphatase activity as a function of age will be reported here.

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The experimental work was carried out at the School of Dentistry, Washington University, St. Louis, Missouri.

MATERIALS AND METHODS

Animals.—All of the 85 animals used in this study were bred and raised in the laboratory. The original breeding stock of C 57 B1/6 mice was obtained from the Roscoe B. Jackson Memorial Laboratory and genetic uniformity was preserved by brother-sister matings. Only unmated males and females were used for the study and the data for each sex were kept separate.

In order to raise the animals under the most uniform environmental conditions, the following conditions were adhered to: temperature of the animal quarters was thermostatically controlled to 75 ± 1 F. and moisture to 50 per cent; twelve hour periods of fluorescent light alternated with twelve hour periods of complete darkness; animal pans and water bottles were sterilized once per week; food (Purina Laboratory Chow) and water were allowed ad libitum.

All of the older animals showed age changes in that the fur turned grey and became less abundant and lustrous. The amount of physical activity was reduced but the mice were alert and responsive. No animal with evidence of gross internal pathologic changes was used for the determinations.

General Procedures.—All animals were sacrificed at the same time of day by means of a blow on the head. After exsanguination the entire liver was removed and blotted free of blood. A small piece of the central lobe was removed for microscopic examination. The remainder was rapidly weighed and homogenized in an all glass grinder using ice cold glass distilled water as diluent. The homogenates were always used immediately after preparation since preliminary experiments indicated that loss of activity occurred even when they were stored at —20 C.

The enzyme reactions were allowed to proceed in test tubes suspended in a constant temperature water bath at 37 C. Incubation time was one hour. One ml. aliquots of the tissue homogenates were added to 7 ml. of the alkaline buffer-substrate mixture and to 14 ml. of the acid buffer-substrate mixture, the compositions of which are given in table 1.

TABLE 1. BUFFER-SUBSTRATE MIXTURES FOR PHOSPHATASE ACTIVITY.

	Final Concentrations	
	Alkaline	Acid
Sodium glycerophosphate*	0.0540 M	0.01080 M
Sodium barbital	0.0277 M	
Magnesium chloride	0.0097 M	
Acetate buffer		0.1146 M
pH	9.45	5.75

*Eastman Kodak # 644 containing 20-28 % alpha salts.

The reactions were stopped by addition of trichloro acetic acid to a final concentration of 10 per cent. After centrifugation aliquots of the supernatant were used for phosphate determination using the classical method of Fiske and SubbaRow (9).

Total nitrogen was determined on a 1 ml. aliquot of each homogenate by a modification of the Conway micro diffusion cell method (5, 17).

RESULTS

The data reported here represent a study of the behavior of total homogenates of the mouse liver toward the substrate sodium glycerophosphate and are expressed in terms of micrograms of phosphorous liberated per gram of wet weight of tissue and as micrograms of phosphorous liberated per milligram total nitrogen per hour of incubation.* In order to secure optimum conditions the effects of hydrogen ion concentration, substrate concentration, enzyme concentration, magnesium activation, and length of incubation were investigated.

*Since the beginning of this work Tsuboi (30) has reported quantitative data on normal and regenerating hepatic tissues of male strain A mice of 2-3 months of age using disodium phenyl phosphate as substrate.

Characteristics of the Enzymes

The pH behavior in the acid and alkaline ranges are shown in figure 1. Acid phosphatase activity rises sharply, reaching a peak at pH 5.75. In the alkaline range the effect is less striking, but a small peak seems to be present at pH 9.45. This is in the contrast to the observation by Tsuboi (30) who was not able to find a pH optimum in the extreme alkaline region but for subsequent experiments selected pH 9.2 as the pH which would probably give optimum or near optimum results.

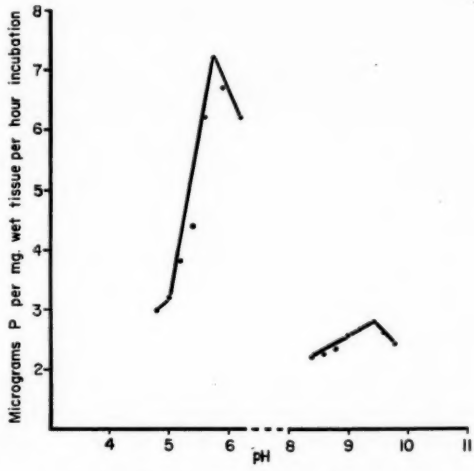

FIG. 1. Effect of pH on phosphatase activity.

The effect of varying the substrate concentration at these pH optima was studied (fig. 2). In both enzyme reactions the velocity depends on the substrate concentration over the first part of the course. Later, the rate of change becomes independent of substrate concentration. Based on these data a final substrate concentration of 0.0540 M for the alkaline phosphatase and 0.1080 M for the acid phosphatase were selected for use in subsequent experiments.

Determinations of total nitrogen were made on aliquots of each tissue homogenate. The results are expressed in terms of mg. N per ml. of homogenate and are used here to represent the concentration of enzyme. Typical results of varying the concentration of enzyme while keeping other factors constant are given in table 2. These results indicate that at pH 9.45 a linear relationship exists over a wide range of concentrations while at pH 5.75 the proportionality exists up to a concentration of between 0.87 and 1.74 mg. N per ml. of homogenate.

The velocity of reaction with relation to the length of incubation at 37 C. is shown in figure 3. The rate remains constant for at least two hours in the case

of the alkaline enzyme and for one hour in the case of the acid phosphatase.

It has often been reported that alkaline phosphatase is activated by magnesium and other divalent ions. The degree of activation is influenced by the purity of the preparation, the substrate used, and the pH of the medium (27). The findings here are in agreement with previous reports. Activity increases with an increase in magnesium up to a concentration of 0.0097 M, then declines and levels off.

Effect of Age on Phosphatase Activities

Quantitative data for liver alkaline and acid phosphatase activity were collected for 15 groups of mice ranging in age from 1 month to 24 months. With the exception of 12 and 21 months, each age group consisted of 6 animals, 3 males and 3 females. The mean values for the pooled male and female data are given in table 3. Also given is the total nitrogen content in terms of micrograms N per mg. wet weight of tissue.

1. Alkaline phosphatase.—During the first twelve and from the seventeenth to the twenty-fourth months there are month to month variations in the alkaline phosphatase activity, none of which assume statistical significance. For the purpose of analysis of aging changes, the data were pooled into 3 groups and the means and standard deviations

TABLE 2. THE RELATIONSHIP BETWEEN ALKALINE AND ACID PHOSPHATASE ACTIVITY AND CONCENTRATION OF NITROGEN.

Mg. Tissue/Ml. Homogenate	Mg. N/Ml. Homogenate	Micrograms P per Mg. N
Alkaline Phosphatase (pH 9.45)		
8.75	0.275	108.06
17.51	0.550	113.22
35.02	1.100	105.81
70.06	2.201	107.74
140.10	4.401	113.22
Acid Phosphatase (pH 5.75)		
6.53	0.218	433.33
13.06	0.435	439.39
26.12	0.870	433.33
52.25	1.740	421.21
104.50	3.480	403.03

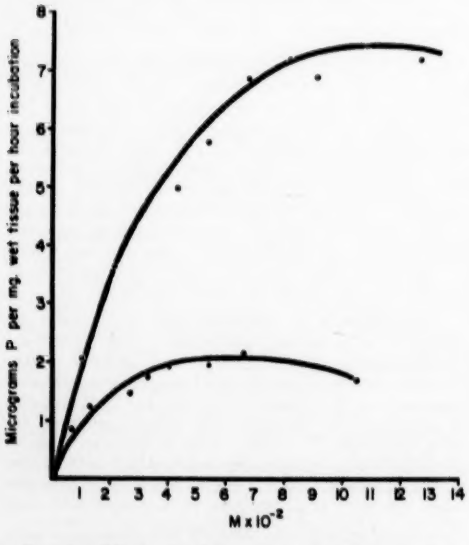

FIG. 2. Effect of substrate concentration on phosphatase activity. Upper curve, acid phosphatase. Lower curve, alkaline phosphatase.

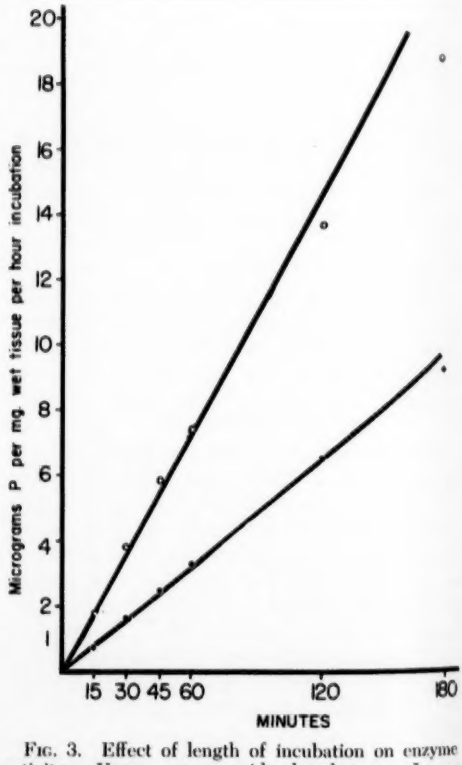

FIG. 3. Effect of length of incubation on enzyme activity. Upper curve, acid phosphatase. Lower curve, alkaline phosphatase.

calculated (table 4). The 1 and 2 month old mice, which can be considered as young animals, were placed in group I. In group II were placed the young adult and adult animals of 3 to 12 months. In the female, at least, this represents the useful breeding period. Animals of 17 to 24 months were considered senile and constituted group III. The data for groups I and III were compared with those for group II and a difference having a P value below 0.01 was considered statistically significant. On this basis it was found that the alkaline phosphatase activities of the young animals (group I) and young adult and adult animals (group II) do not differ from each other. On the other hand, the senile animals of group III show a significant increase in activity. Expressed as micrograms P/mg. of wet weight of tissue/hour of incubation, group III has an activity of 4.31 as compared with 3.29 for group II and 3.46 for group I. Activity calculated in terms of nitrogen content shows the same rise in group III.

2. *Acid phosphatase.*—The monthly data for acid phosphatase activity are given in table 3. As in the case of the alkaline phosphatase, variations in the mean values occur from month to month from 1 to 24 months. Statistical examination of the pooled data (table

TABLE 3. PHOSPHATASE ACTIVITY IN MOUSE LIVER.

Age in Months	No. of Animals	Mg. N/Mg. Wet Tissue	Alkaline Phosphatase		Acid Phosphatase	
			Micrograms P per Mg. Wet Tissue	Micrograms P per Mg. N	Micrograms P per Mg. Wet Tissue	Micrograms P per Mg. N
1	6	.033	3.17 (2.88–3.61)	95 (84–103)	11.96 (11.56–12.34)	358 (342–388)
2	6	.035	3.74 (3.33–4.16)	106 (95–116)	12.90 (11.40–15.22)	383 (346–431)
3	6	.034	3.51 (3.09–3.99)	118 (85–131)	11.54 (10.86–12.63)	373 (332–398)
4	6	.034	3.62 (3.23–4.01)	106 (92–119)	12.87 (11.99–14.14)	373 (355–402)
5	6	.034	3.65 (3.60–4.01)	119 (104–140)	13.29 (11.06–13.77)	387 (349–426)
6	6	.033	3.35 (2.88–3.80)	95 (84–103)	11.83 (10.64–13.84)	358 (342–396)
7	6	.032	3.58 (2.99–3.90)	118 (104–137)	11.96 (10.83–13.05)	379 (361–454)
8	6	.034	3.14 (2.55–3.50)	92 (77–103)	12.93 (11.09–14.90)	361 (327–425)
9	6	.034	3.25 (2.80–3.60)	90 (74–103)	13.47 (11.80–15.30)	420 (388–465)
11	6	.033	2.94 (2.56–3.54)	89 (72–109)	13.30 (12.40–14.50)	400 (364–455)
12	5	.037	2.77 (2.57–2.94)	76 (70–81)	12.84 (11.30–15.80)	371 (312–438)
17	6	.033	4.00 (3.70–4.50)	119 (109–134)	10.45 (9.17–11.99)	322 (278–363)
20	6	.033	4.33 (4.17–4.51)	131 (121–141)	10.25 (9.73–10.78)	321 (314–334)
21	2	.033	4.66 (4.01–5.32)	147 (124–171)	——	——
24	6	.034	4.48 (4.23–5.48)	133 (121–161)	9.70 (6.49–10.81)	288 (203–357)

TABLE 4. PHOSPHATASE ACTIVITY IN MOUSE LIVER; POOLED DATA.

The individual data have been pooled into 3 age groups. For each group are given the mean value and in brackets the standard deviations. Bold data differ significantly, P less than 0.01, from those of group II of the same section.

Group	Age	No. of Animals	Alkaline		Acid	
			Micrograms P per Mg. Wet Tissue	Micrograms P per Mg. N	Micrograms P per Mg. Wet Tissue	Micrograms P per Mg. N
I	Young animals 1–2 months	12	3.46 (0.41)	101 (8.3)	12.43 (1.5)	374 (34)
II	Young adult and adult animals 3–12 months	53	3.29 (0.51)	100 (11.5)	12.71 (1.5)	385 (46)
III	Senile animals 17–24 months	18	4.31 **(0.42)**	129 **(17.7)**	10.18 **(1.6)**	305 **(46)**

TABLE 5. ALKALINE PHOSPHATASE ACTIVITY IN MOUSE LIVER AS A FUNCTION OF SEX. ACTIVITY EXPRESSED AS MICROGRAMS P/MG. WET WEIGHT OF TISSUE/HR. INCUBATION.

Age in Months	Female	Male	Age in Months	Female	Male
1	3.0	3.2	8	3.1	2.6
	3.1	3.2		3.5	2.9
	2.9	3.6		3.3	3.2
2	4.2	4.1	9	3.6	2.8
	3.8	3.5		3.6	2.9
	3.6	3.3		3.5	3.1
3	4.0	3.1	11	2.9	2.6
	3.9	3.2		3.5	2.6
	3.7	3.1		2.9	3.1
4	4.0	3.2	12	2.9	2.6
	3.9	3.4		2.8	2.7
	3.7	3.4		2.8	—
5	4.0	3.8	17	4.5	3.7
	3.9	3.6		4.5	3.8
	3.9	3.8		4.1	3.9
6	3.6	2.9	20	4.5	4.2
	3.7	3.2		4.5	4.2
	3.8	3.1		4.3	4.3
7	3.8	3.0	24	4.4 4.2	—
	3.9	3.6		4.2 4.3	—
	3.9	3.2		4.3 5.5	—

4) reveals that the activities of groups I and II are of roughly the same order of magnitude. The senile animals, however, show a significant decrease in activity when compared with the other groups. This is apparent both in the data calculated in terms of total nitrogen content and as wet weight of tissue.

3. *Influence of sex.*—In studying the spread of data within each month for both the alkaline and acid phosphatase activity, separate plots were made of the data from each sex. With respect to the acid phosphatase, it was immediately apparent that a relationship between sex and enzyme activity is not demonstrable, the activity for one sex being sometimes higher and sometimes lower or of the same order of magnitude as that of the other sex. On the other hand, the data for the alkaline phosphatase are very suggestive of a relationship. With the exception of the 1 month animals, the values for the females are always higher than those of the males (table 5). Statistical analysis of pooled third to twelfth month data (previously classified as the useful breeding period of the female) indicated that the liver alkaline phosphatase activity of the female mouse is significantly higher than that of the male mouse. This is also true for pooled 17 and 20 month data.

In view of this sex difference the data for groups I, II, and III previously discussed were separated according to sex and were re-examined. The possibility suggested itself that the group III males might not show the significant increase in activity which is found when the sexes are lumped if they are compared with the younger females. However this was not found to be so. Group III males show a statistically significant increase in activity when compared with either group I and II females or males.

DISCUSSION

All too few studies of the enzyme activities of tissues and organs as a function of aging have been reported and none have been specifically concerned with the mouse liver. In such reports as are available, no constant pattern of change associated with age has been demonstrated. Thus, for example, in senile human brain tissue acid phosphatase activity increases while glucose metabolism (aerobic and anaerobic) decreases (13, 26). In cattle lenses glycerophosphatase activity seems to be the same with increasing age while hexosediphosphatase activity decreases to about half the value found in young animals (22). The data reported here give evidence that senility in the mouse liver is accompanied by changes in the activity of both the alkaline and acid phosphatases. However the changes are not parallel, an increase occurring in the activity of the alkaline enzyme while the activity of the acid enzyme decreases. Recently Lowry and Hastings (18) have reviewed the existing knowledge of the chemistry of aging tissues. Using their method of deductive histochemistry, they recalculated the data of other authors and, taking them in conjunction with their own data, concluded that a decrease in solids and an increase in water content occurs in many tissues in senescence. In the present study the nitrogen content of the senile animals was found to remain unchanged.

The phosphatases have been found to be widely distributed in mammalian tissues and to be associated with a number of functions. The indications are good that the alkaline phosphatase is associated with calcification mechanisms and the functional activity of the lining cells of the small intestine and proximal convoluted tubule of the kidney (20). With reference to its role in the economy of the liver, some difference of opinion seems to be present. In brief, liver alkaline phosphatase activity has been studied in relation to regeneration of liver parenchyma, protein metabolism, carbohydrate metabolism, secretions of cortical hormones, stress, sex hormones, and pituitary hormones (15, 19, 23, 24, 31). The question of whether the liver secretes alkaline phosphatase into the bile or merely transports it into the bile from other sources has been the subject of many discussions (31).

Although the mouse liver has not been specifically investigated, evidence is present that in mammalian liver a phosphatase is involved in the series of reactions leading from glycogen to free glucose (6). Mathies, Gaebler, and Palm (19) showed that in hypophysectomized, fasted rats, a decrease in liver glycogen is accompanied by an increase in alkaline phosphatase activity. The suggestion was made that the increase in enzyme activity was related to the increased glycolysis. A study of the storage of liver glycogen as related to age

alone was made by Deuel and associates (8). These authors showed that the glycogen content is considerably lower in 24 month old rats than in young animals. In the present study it was noted that alkaline phosphatase activity increases in senility. The possibility exists that this increase in liver phosphatase is related to changes in glycogen metabolism. This is presently being investigated.

The role of the acid phosphatase in the liver cell is still surrounded by mystery. As pointed out by Flexner and Flexner (10), the elucidation of the function of this enzyme presents a challenge to the student of cellular metabolism. A suggestion was made earlier that in tissues having low alkaline phosphatase activity and high acid phosphatase activity, the acid enzyme is associated with an active glycolytic mechanism (20).

Mention should be made here of the relationship between sex and the liver alkaline phosphatase activity. It was pointed out earlier that during the period of sexual maturity the activity in the liver is higher in female than in male mice. Reports have appeared in the literature describing variations in the distribution and level of alkaline phosphatase activity in the female reproductive tract during different phases of the normal cycle as well as in pregnancy and lactation (2, 3, 7, 25, 28, 33). There is also evidence that administered estrogens stimulate alkaline phosphatase activity in the reproductive system (2, 3, 12, 25). Buchwald and Hudson (4) reported that serum and bone phosphatase are affected by estrogen administration. Tuba (29) recently found that the serum alkaline phosphatase levels in virgin female mice of the C 57, C_3 H and A strains are significantly higher than either the male or breeder females of the same strains.

It is possible that the level of liver phosphatase activity is influenced by the titer of circulating estrogen. This theory is supported by the findings of another study by the author (34) in which the enzyme activity was investigated in fetal livers and in post-natal livers at approximately two day intervals in the first 30 days of life. In these young, sexually immature animals no correlation between the sex of the animal and the level of activity could be demonstrated. The possibility should be considered that the apparent higher activity in the female is merely due to a depressing action exerted by the circulating androgens in the male. However this does not seem likely in view of the findings of Kochakian and Fox (14) that neither castration nor testosterone treatment of normal or castrated male mice has any effect on liver alkaline phosphatase activity.

The data in this paper do not support the finding of Kochakian and Fox (14) that 75 day old male Buffalo-Marsh mice have lower alkaline phosphatase and higher acid phosphatase activity of liver tissue than 155 day old mice. The suggestion can be made that this represents a species difference. The acid to alkaline phosphatase ratio of the liver has been observed to vary with different strains and species (14). Thus, a mixed albino strain of mice has an acid to alkaline phosphatase ratio of 2 to 1; Buffalo-Marsh mice, 1 to 4 or 20 to 1, depending on the age of the animals (14); A strain mice, 8½ to 1 (29); C 57 B1/6 mice vary from 4.6 to 1 to 2.2 to 1, depending on the age of the animals (present study). Species differences have been reported for mouse serum alkaline phosphatase levels (29).

These differences could also be due to the use of different substrates. Although there is still disagreement as to whether mammalian tissues contain a single alkaline phosphatase which can utilize many different substrates or whether each substrate requires a different phosphatase, the variation in the data could represent the preferential use of a substrate by a species.

Lastly, it can be suggested that these differences are simply due to sampling error. Variations of activity occur with each age group. If only a small sample for each age is used for a study, the false impression of an age difference would result.

SUMMARY

A study was made of the alkaline and acid phosphatase activity of the liver of 85 C 57 B1/6 mice of different ages from 1 month to 24 months.

Alkaline phosphatase activity remains relatively constant in young, young adult, and adult animals but rises significantly in senile animals of 17 to 24 months of age. The activity is always higher in virgin females than in males.

Acid phosphatase activity also remains constant in the young and adult mice. However in the senile animals it shows a significant decrease in activity.

The total nitrogen of the liver does not change in senility.

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THE TOTAL NICOTINIC ACID AND PYRIDINE NUCLEOTIDE CONTENT OF HUMAN AORTIC TISSUE*

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PREVIOUS investigations from this laboratory (5) have revealed the presence in human aortic tissue of various coenzyme dependent dehydrogenases. In the present communication the results of determination of the total nicotinic acid and phospho-pyridine nucleotide concentrations in the aortic tissue will be reported.

METHODS

Sixty-five samples of human thoracic aortas were obtained fresh at autopsy at the St. Louis City Morgue† and immediately after removal placed in sterile beakers immersed in crushed ice. The age of the individuals from whom the samples were derived ranged between 2 weeks and 82 years. After removal of the adventitia the aortic tissue was rinsed rapidly with water to remove adherent blood and dried with filter paper; samples of the vessel (intima-media segments) were selected which showed the least degree of arteriosclerotic changes. The tissue was then homogenized in a pyrex grinder with 9 volumes of chilled 0.1 M phosphate buffer, pH 7.4, making a 10 per cent homogenate. During the homogenization the tube of the grinder was kept immersed in ice water.

Total nicotinic acid. The procedure of Dann and Handler (4) was employed for hydrolysis of the pyridine nucleotides, extraction of the nicotinic acid from the tissue, and color development. Ten to 15 ml. of homogenate, corresponding to 1.0 to 1.5 Gm. of wet tissue, were used for each extraction, and 5.0 ml. aliquots of the 25.0 ml. extract for color development. A freshly prepared 4 per cent solution of cyanogen bromide (2) was employed instead of the 3 per cent solution used by Dann and Handler. The metol reagent supplied by the Eastman Kodak Company (Elon) was found to give the lowest blank value of various preparations tested. The color intensities of the samples were read in a Coleman No. 14 spectrophotometer at a wave length of 400$\mu\mu$. A set of nicotinic acid standards was run with each series of determinations.

Experiments were conducted to test the completeness of the nicotinic acid extraction from the tissue under the conditions used. For this purpose the sediment present after the usual hydrochloric acid extraction of a homogenate sample was again subjected to extraction with the same volume of hydrochloric acid as used initially. This second extraction failed to yield nicotinic acid in excess of the calculated content in the sediment.

The average error in analysis of pure nicotinic acid solutions was 6.0 per cent. For duplicates of the same tissue extract and for samples of different extracts from the same tissue homogenate the average errors were 6.1 and 6.2 per cent, respectively. The nicotinic acid values observed by determinations on homogenates prepared from different tissue samples of the same aorta showed a greater degree of variation. The average difference in nicotinic acid content between such adjacent parts of an aorta was 12.2 per cent. Nicotinic acid added to the homogenates before hydrolysis was recovered quantitatively.

Pyridine nucleotides. For determination of the phospho-pyridine nucleotide content of the aortic tissue the fluorometric methyl ethyl ketone alkali condensation method of Burch (3) was employed, using a standard solution of N^1-methyl nicotinamide° as reference. Because of the instability of the pyridine nucleotides in the tissue great care was exercised to keep the samples constantly refrigerated before and during the homogenization. For each determination 1.0 ml. of homogenate was pipetted into a test tube, to which was immediately added 50 cu. mm. of the ceric sulfate-nicotinamide reagent. The content of the tube was carefully mixed, after which the protein was precipitated by addition of 0.10 ml. 50 per cent trichloroacetic acid solution, prepared from redistilled trichloroacetic acid crystals. The tube was cooled in ice water and centrifuged for five minutes. Fifty cu. mm. of the supernatant solution, representing 4.35 mg. of wet tissue, were used for development of fluorescence. The fluorescence measurements were performed in a Farrand photoelectric fluorometer, using 3.0 ml. cuvettes. The manganese chloride-methyl ethyl ketone solution, N^1-methyl nicotinamide standard, and quinine sulfate reference solution were prepared fresh daily. Since the fluorometric method does not permit a distinction between triphospho- and diphospho-pyridine nucleotides, the observed values are reported as diphospho-pyridine nucleotide (DPN) concentrations.

The proportionality observed between the DPN content of the samples and the fluorometric readings may be seen from figure 1, in

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°The N^1-methyl nicotinamide was generously supplied by Hoffmann-La Roche, Inc.

which the results of analyses of a commercial cozymase I preparation (Sigma Chemical Company, St. Louis) are presented. Solutions in water were prepared from this compound, containing 1, 2, 3, 4, 5, 6, 7, and 8 micrograms per ml. of the cozymase sample. Fifty cu. mm. of each of these solutions were then used for development of fluorescence. A comparison of the fluorescence intensities with that of a N'-methyl nicotinamide standard revealed a cozymase content of the preparation of 75 per cent (reading of N'-methyl nicotinamide standard, 50 cu. mm. of 1 microgram/ml. solution:39; reading of blank:9).

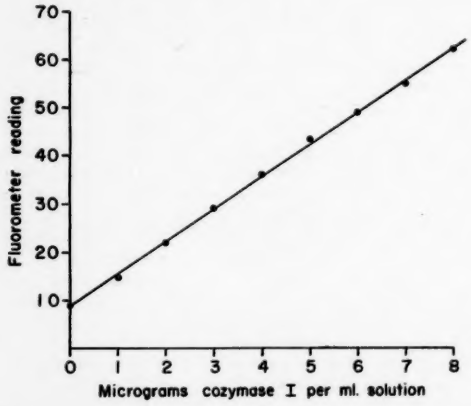

FIG. 1. Fluorometer standard curve for solution of cozymase I (75 per cent purity) in water. Volume of solution used for fluorometric determination:50 cu. mm. Reading of reference standard (50 cu. mm. N'-methyl nicotinamide solution, 1 microgram per ml.) :39. Reading of blank:9.

The average error in analysis of cozymase solutions was 1.7 per cent, and in analysis of aliquots of the same trichloroacetic acid supernatant, 3.7 per cent. Determinations of the DPN content of different trichloroacetic acid extracts prepared from the same homogenate showed a mean variation of 4.1 per cent. The DPN values encountered by varying the amount of tissue represented in the sample for fluorescence development between 2.18 and 4.35 mg. were within the experimental error of the procedure.

RESULTS

The results of determinations of the total nicotinic acid content of 65 human aortic samples are shown in table 1. The table further contains the observed DPN values for 40 samples. It will be seen that the total nicotinic acid concentration of the samples varied between 10.2 and 33.0 micrograms per Gm. wet tissue, with an average value of 19.2 micrograms/Gm. For the DPN determinations the mean value observed was 25.8 micrograms per Gm. wet tissue (range 4.9 to 51.8).

A tendency was noted for the total nicotinic acid content of the aortic tissue to decrease with the age of the subjects from whom the samples were derived. A calculation of the coefficient of correlation: Age/total nicotinic acid concentration, revealed an r value of -0.37 ($t = 3.17$).

In order to investigate the rate of breakdown of the pyridine nucleotides in the aortic tissue during storage of the samples various experiments were carried out, the results of which are listed in table 2. It will be seen from the table that a rapid destruction of DPN occurred at 37 C. The decrease averaged 27 per cent after ½ hour's incubation, 46 per cent after 1 hour, 51 per cent after 2 hours, and 72 per cent after 24 hours. Following storage of the tissue at 4 C. the mean decrease in DPN concentration observed after 24 hours was 48 per cent. In a few instances, where the studies were continued and observations of the DPN content made after two to five days of storage, no further significant reduction in the pyridine nucleotide concentration was noted. These findings suggest that the enzymatic breakdown of the pyridine nucleotides comes to an end as the result of the destruction of the enzyme (DPNase) itself.

A support for this contention was obtained in an experiment on a sample of intact aortic tissue (#45) for which a decrease in the DPN content from 17.0 to 14.1 micrograms/Gm. wet tissue had been found following five days' storage of the sample at 4 C. When after this period a homogenate of the tissue was prepared and incubated for 2 hours at 37 C. the DPN value of the sample remained unchanged (14.0 microgram/Gm.). A homogenate of another, fresh, aortic sample (#28) was now made. This homogenate showed an initial DPN value of 30.2 micrograms/Gm., which after two hours' incubation at 37 C. was found to decrease to 15.1 micrograms/Gm. However, when an equal volume of the fresh

TABLE 1. TOTAL NICOTINIC ACID AND PYRIDINE NUCLEOTIDE CONTENT OF HUMAN AORTIC TISSUE.

Sample No.	Sex	Age in Years	Total Nicotinic Acid Micrograms/Gm. Wet Tissue	Pyridine Nucleotides* Micrograms/Gm. Wet Tissue
1	M	0.04	27.3	40.8
2	F	0.2	17.7	29.1
3	F	0.4	33.0	36.3
4	M	0.7	29.4	23.4
5	F	0.8	22.8	
6	M	1	13.7	
7	F	2	28.5	
		Mean	24.6	
8	F	24	14.3	27.1
9	M	28	29.7	
10	M	29	14.2	
		Mean	19.4	
11	M	30	22.6	44.2
12	M	30	15.0	24.2
13	M	30	19.2	
14	M	35	20.8	
15	M	37	22.8	22.0
		Mean	20.0	
16	M	42	16.5	
17	M	43	15.9	
18	M	44	18.9	
19	M	45	22.6	51.8
20	M	46	22.5	
21	M	47	15.4	
22	M	49	32.5	
23	M	49	19.7	
24	M	49	23.4	24.2
25	F	49	19.7	10.9
		Mean	20.7	
26	M	50	18.0	26.3
27	F	50	15.0	31.7
28	F	51	15.0	30.2
29	M	52	21.3	
30	M	52	21.4	14.3
31	M	52	26.7	48.5
32	M	53	14.8	38.6
33	M	57	10.2	
34	F	58	14.8	
35	M	58	11.9	
36	M	58	24.6	35.9
37	M	59	16.0	
38	M	59	19.0	27.0
		Mean	17.6	

TABLE 1. TOTAL NICOTINIC ACID AND PYRIDINE NUCLEOTIDE CONTENT OF HUMAN AORTIC TISSUE (Continued)

Sample No.	Sex	Age in Years	Total Nicotinic Acid Micrograms/Gm. Wet Tissue	Pyridine Nucleotides* Micrograms/Gm. Wet Tissue
39	M	60	11.2	15.1
40	M	60	19.1	
41	M	60	21.4	
42	M	60	12.1	43.5
43	M	61	16.5	22.3
44	M	62	18.3	17.7
45	M	62	14.3	17.0
46	M	64	16.6	
47	M	64	13.5	
48	M	64	17.2	26.1
49	M	65	24.4	
50	M	65	21.2	4.9
51	F	65	22.4	18.9
52	M	67	15.7	22.7
53	F	67	15.6	19.6
54	M	68	11.3	10.9
55	M	69	23.1	36.3
Mean			17.3	
56	M	70	16.5	22.7
57	M	71	16.3	37.1
58	F	71	15.8	18.0
59	M	72	16.5	
60	M	72	21.7	27.6
61	M	75	23.1	32.9
62	M	75	21.7	14.4
63	M	76	14.5	
64	F	79	24.2	20.6
Mean			18.9	
65	M	82	11.6	7.9
Grand Mean			19.2	

*As explained in the text the DPN values observed under the existing conditions for analysis of aortic tissue must be assumed to be lower than the cozymase concentrations present in the tissue in vivo.

homogenate from sample #28 (30.2 micrograms/Gm.) was added to the homogenate from sample #45 (14.1 micrograms/Gm.) the DPN content or the mixed homogenate after two hours' incubation at 37 C. was found to have decreased to 8.7 micrograms/Gm.

No change in the total nicotinic acid content of the tissue was noted following storage of the aortic samples at 4 C. for 24 hours.

DISCUSSION

The total nicotinic acid values observed for human aortic tissue in the present investigation are about 30, 50, and 200 per cent, respectively, of the nicotinic acid concentrations reported by Bandier (1) for the human liver, kidney, and brain. Since a rapid breakdown of the pyridine nucleotides was found to occur even during brief storage of the tissue samples,

TABLE 2. DPN -ASE ACTIVITY OF HUMAN AORTIC TISSUE

Values represent micrograms of DPN per Gm. wet tissue observed after various periods of storage.

Sample No.	Preparation	Temperature C.	Storage Period in Hours				
			0	½	1	2	24
24	Intact tissue	4	24.2				14.0
25	"	4	10.9				6.3
50	"	4	4.9				3.6
61	"	4	32.9				14.1
66	"	4	51.8				27.0
68	"	4	28.0	25.5	16.2	19.6	15.1
28	"	37	30.2			15.1	
36	"	37	35.9	30.6	20.8	26.4	11.7
39	"	37	15.1	10.9	11.7	9.8	9.8
55	Homogenate	37	36.3	28.8	20.0	18.5	
66	"	37	51.8	26.5	17.9	7.6	7.6
67	"	37	26.3	18.9	18.4	13.6	

the DPN values found in the present study cannot be assumed to represent the true cozymase concentrations occurring in the aortic tissue. The values are included in the publication merely to supply information about actually observed tissue concentrations for the coenzyme. It should be noted that the DPN values found on the average account for only about 25 per cent of the observed total nicotinic acid concentrations. Whether all the nicotinic acid found on acid hydrolysis of aortic tissue represents material initially present as pyridine nucleotides cannot be decided without access to human tissue immediately after death.

The observed decrease in the nicotinic acid content of the aortic tissue with age may be due to an actual decrease in the nicotinic acid concentration of the active aortic tissue, to partial displacement of normal structures by pathologic tissue, or to a combination of such changes. An evaluation of the relative significance of the suggested causes for the reduction in the nicotinic acid content of the samples with age is hardly possible with the technique available at the present time.

SUMMARY

Determinations were made by the method of Dann and Handler of the total nicotinic acid content of 65 samples of human thoracic aortas, derived from individuals ranging in age between 2 weeks and 82 years. An average nicotinic acid concentration of 19.2 micrograms per Gm. wet tissue was observed. A tendency was found for the nicotinic acid content of the samples to decrease with the age of the subjects. No change in nicotinic acid concentration of the samples was noted on storage of the tissue at 4 C.

Measurements of the phospho-pyridine nucleotide content of the aortic tissue were performed on 40 of the samples, using the fluorometric procedure of Burch. A mean DPN value of 25.8 micrograms per Gm. wet tissue was found. Since the presence of an active DPN-ase was demonstrated in the arterial tissue the observed DPN values must be assumed to be lower than the cozymase concentrations present in the aortic tissue in vivo.

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THE RIBOFLAVIN CONTENT OF HUMAN AORTIC TISSUE*

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Since the nucleotides of riboflavin constitute prosthetic groups of several enzymes associated with tissue respiration, an investigation of the riboflavin content of arterial tissue was considered of importance. The recent introduction by Bessey, Lowry, and Love (2) of an accurate fluorometric procedure for measurement of small concentrations of riboflavin in tissues rendered possible the determination of the free riboflavin + flavin mononucleotide, flavin adenine dinucleotide, and total riboflavin content of a series of samples of human thoracic aortas. For comparative purposes riboflavin determinations on a smaller group of dog aortas were included in the investigation.

METHODS

One hundred samples of human thoracic aortas were obtained fresh at autopsy at the St. Louis City Morgue† and immediately placed in sterile beakers immersed in crushed ice. The age of the individuals from whom the samples were derived ranged between 4 weeks and 83 years. After removal of the adventitia the aortic tissue was rinsed rapidly with water to remove adherent blood. The water on the surface of the tissue was subsequently removed by means of filter paper, and samples of the vessel (intima-media segments) were selected which showed the least degree of arteriosclerotic changes.

Eight-tenth to 1.2 Gm. of aortic tissue was homogenized with 24 volumes of its weight of ice-cold, twice glass-distilled water, using a Pyrex homogenizer. During the homogenization the homogenizer was immersed in ice water. The homogenate was transfered quantitatively to an Erlenmeyer flask of amber glass containing 25 ml. of chilled, 20 per cent trichloroacetic acid solution (prepared from redistilled trichloroacetic acid crystals), giving a final trichloroacetic acid concentration of 10 per cent. The contents of the Erlenmeyer flask were shaken, and approximately five minutes allowed for precipitation of the protein. Both before and after the transfer of the homogenate the Erlenmeyer flask was maintained in a basin of ice water.

The sample was then transferred to a 50 ml. centrifuge tube. In order to obtain a crystal clear supernatant it was found necessary to let the sample stand for about one hour before centrifugation. During this period the centrifuge tube, together with its metal carrier, was placed in the freezing unit of a refrigerator to keep the acid hydrolysis of flavin adenine dinucleotide as low as possible. Care was taken to avoid solidification of the contents of the tube. The sample was then centrifuged for ten minutes at 3000 r.p.m., after which the supernatant was syphoned off and placed in an amber test tube immersed in ice water. One ml. of the supernatant was used for determination of the apparent riboflavin content of the sample, and a similar volume employed, after hydrolysis, for determination of total riboflavin. The analyses were carried out in detail as described by Bessey, Lowry, and Love (2), using a Farrand photoelectric fluorometer, and the appropriate formulas given by these authors employed for calculation of the various riboflavin fractions. All determinations were carried out in duplicate.

Experiments were conducted to test the completeness of the riboflavin extraction from the aortic tissue under the conditions used. For this purpose the protein precipitate resulting from centrifugation of the trichloroacetic acid treated homogenate was suspended in the same volume of 10 per cent trichloroacetic acid as used initially, and, after stirring, again centrifuged. The supernatant solution obtained from this second centrifugation failed to yield riboflavin in excess of the calculated content of the sediment. It was further ascertained that riboflavin added to the homogenate before precipitation with trichloroacetic acid could be recovered quantitatively.

The average error in analysis of the total riboflavin content of the same supernatant was established by carrying out 10 determinations on a hydrolyzed tissue extract. The mean error observed in these analyses was 3.1 per cent.

Five dog aortas for riboflavin determination were obtained at the Department of Surgery, Washington University.* The thoracic aortas were removed from the anesthetized animals and immediately chilled with carbon dioxide snow. The subsequent preparation of the tissue and the riboflavin de-

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*The assistance of Dr. H. R. Butcher in obtaining the dog aortas is gratefully acknowledged.

terminations were carried out as described for the human samples.

In the case of all the dog and a few of the human samples, the riboflavin determinations mentioned above were supplemented with determination of the free riboflavin content of the tissue. For these analyses a volume of the initial, non-hydrolyzed, neutralized supernatant solution was shaken with an equal volume of freshly distilled, water saturated, C. P. benzyl alcohol in a glass stoppered measuring cylinder. The benzyl alcohol layer was syphoned off, and 1 volume of the benzyl alcohol extract shaken in another cylinder with 15 volumes of redistilled toluene and 1 volume of water, containing sodium acetate and acetic acid in 0.05 M concentrations. After separation of the solutions the aqueous layer was employed for determination of free riboflavin.

RESULTS

The results of determinations of the concentrations of free riboflavin + flavin mononucleotide, flavin adenine dinucleotide, and total riboflavin in the 100 human aortic samples are shown in table 1. It will be seen from the table that the total riboflavin content of the samples varied between 0.50 and 2.34 micrograms/Gm. fresh tissue, with a mean value of 1.16 micrograms/Gm. It will further be noted that the observed concentrations of free riboflavin + flavin mononucleotide in the present series averaged 0.40 micrograms/Gm. (or 34.8 per cent of the total riboflavin), and the flavin adenine dinucleotide concentration 0.76 micrograms/Gm. (or 65.2 per cent of the total riboflavin).

A comparison was made of the average riboflavin concentrations in aortic tissue for the white and colored men and women separately. In these computations only the 93 subjects above the age of 20 years were considered. Results of these calculations are shown in table 2. It will be seen from the table that the differences between the groups are not significant.

As evidenced from the summarized values presented in table 1, a tendency was noted for the riboflavin content of the aortic tissue to decrease with the age of the subjects from whom the samples were derived. A calculation of the coefficient of correlation: Age/total riboflavin concentration revealed an r value of -0.39 ($t=4.20$, $N=100$). The reduction in the riboflavin content of the aortic tissue with age seemed to affect the free riboflavin + flavin mononucleotide and the flavin adenine dinucleotide fractions to approximately the same extent.

The determinations performed on the 5 dog aortas (table 3) showed concentrations of total riboflavin more than twice as high as the average value observed for the 100 human aortic samples. As seen from table 3, the mean concentrations and the percentage values of free riboflavin, flavin mononucleotide, free riboflavin + flavin mononucleotide, flavin adenine dinucleotide, and total riboflavin observed in analysis of dog aortic tissue were, respectively, 0.14 (5.9 per cent), 0.12 (6.1 per cent), 0.26 (11.0 per cent), 2.31 (89.0 per cent), and 2.57 micrograms/Gm. fresh tissue. It should be noted that the flavin adenine dinucleotide fraction in the dog samples averaged 89.0 per cent of the total riboflavin found in the tissue, as compared with a mean value of 65.2 per cent observed for the human aortas. In the case of the 3 human aortic samples, in which separate determinations of free riboflavin and flavin mononucleotide were performed, the average concentrations of these riboflavin fractions valued, respectively, 0.18 micrograms/Gm. (18.7 per cent of total riboflavin), and 0.11 micrograms/Gm. (10.4 per cent of total riboflavin). Although the absolute concentrations of free riboflavin and flavin mononucleotide observed for human tissue thus were of the same order of magnitude as those found in the analyses of the dog aortas, the percentage proportions of these fractions of the total tissue riboflavin were higher than in the case of the dog aortas.

It has been shown by Ochoa and Rossiter (4) and by Bessey, Lowry, and Love (2) that various animal tissues contain enzymes capable of splitting flavin adenine dinucleotide. The investigations by Ochoa and Rossiter were performed on liver slices, and on liver and brain homogenates from rats, maintained at 38 C. in buffer solution of pH 8.4. After three hours' incubation of the samples an average decrease in the flavin adenine dinucleotide concentration of 72 per cent was found in the case of the liver slices, whereas in the experiments with liver and brain homogenates the decrease valued only 36 and 7 per cent, respectively. Bessey, Lowry, and Love (2) in similar studies on rat kidney preparations found no measurable decrease in the flavin adenine dinucleotide concentration of

TABLE 1. THE CONCENTRATIONS OF FREE RIBOFLAVIN + FLAVIN MONONUCLEOTIDE, FLAVIN ADENINE DINUCLEOTIDE, AND TOTAL RIBOFLAVIN IN HUMAN AORTIC TISSUE.

Sample No.	Sex	Race	Age in Years	Free Riboflavin + Flavin Mononucleotide		Flavin Adenine Dinucleotide		Total Riboflavin
				Microgr./Gm. Wet Tissue	% of Total Riboflavin	Microgr./Gm. Wet Tissue	% of Total Riboflavin	Microgr./Gm. Wet Tissue
1.	M	C	0.08	0.39	31	0.85	69	1.24
2.	M	C	0.34	0.54	52	0.50	48	1.04
3.	F	C	0.50	0.93	45	1.11	55	2.04
4.	F	C	0.58	0.94	40	1.40	60	2.34
5.	M	C	2	0.47	26	1.32	74	1.79
6.	M	C	2	0.36	36	0.65	64	1.01
Mean				0.61	38.2	0.97	61.6	1.58
7.	F	W	17	0.43	35	0.79	65	1.22
8.	M	C	22	0.38	26	1.07	74	1.45
9.	F	C	24	0.18	12	1.35	88	1.53
10.	F	W	26	0.53	38	0.86	62	1.39
11.	M	W	27	0.52	42	0.72	58	1.24
12.	M	W	28	0.28	18	1.27	82	1.55
Mean				0.38	27.3	1.05	72.7	1.43
13.	F	W	32	0.30	27	0.82	73	1.12
14.	M	C	33	0.20	20	0.81	80	1.01
15.	F	W	34	0.37	19	1.52	81	1.89
16.	M	W	34	0.39	36	0.68	64	1.07
17.	M	C	38	0.78	35	1.46	65	2.24
18.	F	C	39	0.48	60	0.32	40	0.80
19.	M	W	39	0.38	34	0.72	66	1.10
Mean				0.42	33.0	0.90	67.0	1.32
20.	M	W	41	0.53	43	0.71	57	1.24
21.	M	W	42	0.44	28	1.12	72	1.56
22.	M	W	43	0.33	31	0.72	69	1.05
23.	M	W	43	1.52	83	0.32	17	1.84
24.	M	C	44	0.49	35	0.89	65	1.38
25.	F	C	44	0.29	19	1.20	81	1.49
26.	F	W	45	0.32	36	0.58	64	0.90
27.	F	W	45	0.42	31	0.95	69	1.37
28.	M	W	47	0.49	29	1.19	71	1.68
29.	M	W	48	0.12	17	0.59	83	0.71
30.	M	W	48	0.34	29	0.81	71	1.15
Mean				0.48	34.8	0.83	65.2	1.31
31.	F	W	50	0.23	18	1.03	82	1.26
32.	M	C	50	0.38	41	0.55	59	0.93
33.	M	W	50	0.34	37	0.57	63	0.91
34.	F	W	50	0.36	48	0.39	52	0.75

TABLE 1. THE CONCENTRATIONS OF FREE RIBOFLAVIN + FLAVIN MONONUCLEOTIDE, FLAVIN ADENINE DINUCLEOTIDE, AND TOTAL RIBOFLAVIN IN HUMAN AORTIC TISSUE (Continued).

Sample No.	Sex	Race	Age in Years	Free Riboflavin + Flavin Mononucleotide		Flavin Adenine Dinucleotide		Total Riboflavin
				Microgr./Gm. Wet Tissue	% of Total Riboflavin	Microgr./Gm. Wet Tissue	% of Total Riboflavin	Microgr./Gm. Wet Tissue
35.	F	W	51	0.24	19	1.03	81	1.27
36.	F	W	51	0.39	25	1.14	75	1.53
37.	M	W	52	0.27	22	0.95	78	1.22
38.	M	W	53	0.32	32	0.68	68	1.00
39.	M	W	53	0.40	32	0.84	68	1.24
40.	M	W	54	0.29	19	1.20	81	1.49
Mean				0.32	29.3	0.84	70.7	1.16
41.	M	W	55	0.26	25	0.78	75	1.04
42.	M	C	55	0.24	21	0.90	79	1.14
43.	M	W	55	0.23	23	0.68	77	0.89
44.	M	W	55	0.41	43	0.55	57	0.96
45.	M	C	56	0.22	29	0.53	71	0.75
46.	F	C	57	0.24	17	1.15	83	1.39
47.	F	C	57	0.87	54	0.74	46	1.61
48.	M	C	58	0.33	41	0.47	59	0.80
49.	M	W	58	0.20	23	0.66	77	0.86
50.	M	W	58	0.35	26	1.00	74	1.35
51.	M	C	58	0.08	5	1.39	95	1.47
52.	M	C	59	0.43	43	0.57	57	1.00
53.	M	W	59	0.25	28	0.66	72	0.91
54.	M	W	59	0.24	45	0.29	55	0.53
55.	M	W	59	0.37	33	0.76	67	1.13
56.	M	W	59	0.24	18	1.11	82	1.35
57.	M	C	59	0.60	41	0.85	59	1.45
Mean				0.33	30.2	0.77	69.8	1.10
58.	M	W	60	0.26	26	0.73	74	0.99
59.	M	W	60	0.54	46	0.64	54	1.18
60.	M	W	60	0.43	29	1.04	71	1.47
61.	M	W	60	0.30	31	0.68	69	0.98
62.	M	W	60	0.60	41	0.86	59	1.46
63.	M	W	60	0.46	52	0.43	48	0.89
64.	F	C	60	0.38	38	0.63	62	1.01
65.	M	W	61	0.43	53	0.38	47	0.81
66.	M	W	62	0.45	36	0.80	64	1.25
67.	M	W	62	0.39	35	0.72	65	1.11
68.	F	W	62	0.51	75	0.17	25	0.68
69.	F	C	62	0.26	35	0.48	65	0.74
70.	F	W	63	0.36	17	1.75	83	2.11
71.	M	W	63	0.56	47	0.64	53	1.20
72.	M	W	64	0.14	12	1.00	88	1.14
73.	M	W	64	0.27	44	0.50	56	0.90
Mean				0.46	38.6	0.66	61.4	1.12

TABLE 1. THE CONCENTRATIONS OF FREE RIBOFLAVIN + FLAVIN MONONUCLEOTIDE, FLAVIN ADENINE DINUCLEOTIDE, AND TOTAL RIBOFLAVIN IN HUMAN AORTIC TISSUE (Continued).

Sample No.	Sex	Race	Age in Years	Free Riboflavin + Flavin Mononucleotide		Flavin Adenine Dinucleotide		Total Riboflavin
				Microgr./Gm. Wet Tissue	% of Total Riboflavin	Microgr./Gm. Wet Tissue	% of Total Riboflavin	Microgr./Gm. Wet Tissue
74.	M	C	65	0.17	33	0.35	67	0.52
75.	M	W	66	0.27	29	0.67	71	0.94
76.	M	C	66	0.46	46	0.54	54	1.00
77.	M	W	66	0.43	50	0.43	50	0.86
78.	M	W	66	0.24	22	0.84	78	1.08
79.	M	W	66	0.31	37	0.53	63	0.84
80.	F	W	67	0.32	37	0.55	63	0.87
81.	M	C	67	0.67	46	0.80	54	1.47
82.	M	W	68	0.34	36	0.59	64	0.93
83.	F	C	69	0.21	29	0.52	71	0.73
84.	M	W	69	0.32	40	0.47	60	0.79
85.	F	W	69	0.38	29	0.91	71	1.29
Mean				0.34	36.1	0.60	63.9	0.94
86.	M	W	70	0.43	27	1.14	73	1.57
87.	M	C	70	0.35	43	0.47	57	0.82
88.	M	C	70	0.50	41	0.73	59	1.23
89.	M	W	71	0.33	31	0.73	69	1.06
90.	F	W	72	0.47	54	0.40	46	0.87
91.	M	W	72	0.29	24	0.93	76	1.22
92.	F	W	72	0.09	16	0.46	84	0.55
93.	M	W	73	0.30	37	0.50	63	0.80
94.	M	W	73	0.12	13	0.91	87	1.03
95.	F	W	74	0.44	51	0.42	49	0.86
96.	F	W	74	0.30	27	0.83	73	1.13
97.	F	W	75	0.40	43	0.54	57	0.94
98.	F	W	75	0.36	72	0.14	28	0.50
99.	M	W	78	0.90	58	0.66	42	1.56
Mean				0.38	38.3	0.63	61.7	1.01
100.	M	W	83	0.57	61	0.23	29	0.80
Grand mean				0.40	34.8	0.76	65.2	1.16

intact tissue maintained for 30 minutes at 38 C., or of kidney tissue homogenate kept for the same period of time at 10 C. In contrast to this the flavin adenine dinucleotide concentration of another kidney homogenate maintained at 38 C. after 30 minutes' incubation showed a decrease of 41 per cent, and, after 1 hour, a decrease of 60 per cent.

In view of these observations a study was undertaken to investigate whether a breakdown of flavin adenine dinucleotide occurred in intact human and dog aortic tissue maintained at 38 C. For this purpose sterile samples of a human and a dog aorta were placed in test tubes in a thermostat at 38 C. The tubes were party immersed in water in

TABLE 2. MEAN RIBOFLAVIN CONCENTRATIONS IN AORTIC TISSUE FOR SUB-GROUPS OF WHITE AND COLORED MEN AND WOMEN.

Values represent micrograms/Gm. wet tissue.

Group	N.	Free Riboflavin + Flavin Mononucleotide	Flavin Adenine Dinucleotide	Total Riboflavin
Men, white	50	0.39	0.73	1.12
Men, colored	16	0.40	0.77	1.17
Women, white	19	0.36	0.76	1.12
Women, colored	8	0.36	0.80	1.16

TABLE 3. THE CONCENTRATION OF FREE RIBOFLAVIN, FLAVIN MONONUCLEOTIDE, FLAVIN ADENINE DINUCLEOTIDE, AND TOTAL RIBOFLAVIN IN DOG AND HUMAN AORTIC TISSUE.

Sample No.	Species	Free Riboflavin		Flavin Mononucleotide		Free Riboflavin + Flavin Mononucleotide		Flavin Adenine Dinucleotide		Total Riboflavin
		Microgr./ Gm. Wet Tissue	% of Total Riboflavin	Microgr./ Gm. Wet Tissue	% of Total Riboflavin	Microgr./ Gm. Wet Tissue	% of Total Riboflavin	Microgr./ Gm. Wet Tissue	% of Total Riboflavin	Microgr./ Gm. Wet Tissue
1.	Dog	0.16	7.3	0.19	8.6	0.35	15.9	1.85	84.1	2.20
2.	Dog	0.14	4.9	0.04	1.4	0.18	6.3	2.68	93.7	2.86
3.	Dog	0.19	8.5	0.13	5.8	0.32	14.3	1.91	85.7	2.23
4.	Dog	0.05	1.5	0.11	3.3	0.16	4.8	3.16	95.2	3.32
5.	Dog	0.16	7.1	0.15	6.6	0.31	13.7	1.96	86.3	2.27
Mean		0.14	5.9	0.12	5.1	0.26	11.0	2.31	89.0	2.57
35.	Human	0.18	14.1	0.06	4.7	0.24	18.8	1.03	81.2	1.27
38.	Human	0.10	10.0	0.22	22.0	0.32	32.0	0.68	68.0	1.00
80.	Human	0.28	32.1	0.04	4.6	0.32	36.7	0.55	63.3	0.87
Mean		0.18	17.3	0.10	9.6	0.28	26.9	0.76	73.1	1.04

a closed glass container to insure saturation with water vapor. Determinations of the free riboflavin, flavin mononucleotide, and flavin adenine dinucleotide concentrations of the tissue were performed at the onset of the experiment, and after 14 to 33 hours' maintenance at thermostat temperature. The results of these analyses are presented in table 4 and show a notable decrease of the flavin adenine dinucleotide content, with an increase in the free riboflavin + flavin mononucleotide fraction. The observed decrease in the flavin adenine dinucleotide content of the aortic tissue indicates the presence of an enzyme or enzymes capable of splitting this riboflavin fraction.

A comparison was further made in the case of the human aortic samples of the correlation between the time elapsed from the death of the subjects to the removal of the aortas at autopsy and the relative percentages of the free riboflavin + flavin mononucleotide and flavin adenine dinucleotide fractions found on analysis of the tissue. The results of this comparison are shown in table 5. It will be seen from the table that no significant difference in the percentage values of these riboflavin fractions was observed between human aortas

TABLE 4. EFFECT OF INCUBATION OF INTACT HUMAN AND DOG AORTIC TISSUE AT 38 C. ON CONCENTRATIONS OF VARIOUS RIBOFLAVIN FRACTIONS.

Values represent micrograms/Gm. wet tissue.

	Human Aorta No. 38		Dog Aorta No. 5	
	Original*	After 23 Hours' Incubation	Original	After 14 Hours' Incubation
Free Riboflavin	0.10	0.53	0.16	0.68
Flavin Mononucleotide	0.22	0.11	0.15	0.54
Flavin Adenine Dinucleotide	0.68	0.50	1.96	1.23
Total Riboflavin	1.00	1.14	2.27	2.45

*Original analyses performed six hours after death of subject, following four hours' maintenance of body at refrigeration temperature in morgue and one hour's storage of aortic sample in beaker immersed in crushed ice.

obtained shortly after the death of the subjects and samples removed from the body several hours after the individuals had expired. Since the bodies were kept refrigerated until the performance of the autopsies this finding would seem to indicate that the enzymatic splitting of flavin adenine dinucleotide in human aortic tissue proceeds at a slow rate at refrigeration temperature.

TABLE 5. CORRELATION BETWEEN LENGTH OF TIME INTERVAL FROM DEATH OF SUBJECTS TO PROCUREMENT OF AORTIC SAMPLES AND PERCENTAGE VALUES OF FLAVIN ADENINE DINUCLEOTIDE IN AORTIC TISSUE.

Time From Death to Procurement of Aortic Samples (Hours)	No. of Samples	Flavin Adenine Dinucleotide Content of Aortic Tissue in % of Total Riboflavin Concentration
0– 1	14	67
2– 3	12	72
4– 5	11	63
6– 8	10	57
9–12	12	63
13–16	14	65
17–20	27	64

DISCUSSION

No reports are available in the literature concerning the concentration of riboflavin in human and animal arterial tissue. The results of riboflavin analyses of various human organs have shown an average concentration of total riboflavin in skeletal muscle of about 2 micrograms/Gm. fresh tissue (1, 5, 6) and of about 11 micrograms/Gm. liver tissue (3, 5, 6). For heart, lung, kidney, and brain the mean riboflavin concentrations reported are, respectively, 6, 1.5, 9, and 2 micrograms/Gm. fresh tissue (3, 5, 6). In view of the low respiratory rate of aortic tissue compared with that of most other organs the riboflavin values for arterial tissue observed in the present study must be considered as being rather high.

In the present investigation a higher percentage content of flavin adenine dinucleotide was found in analyses of dog aortic tissue than in the human aortic samples. Since an enzyme capable of splitting flavin adenine dinucleotide was demonstrated in the aortic tissue it is reasonable to ascribe the lower percentage values for flavin adenine dinucleotide observed in the case of the human samples to a partial decomposition of this compound occurring between the supervention of death and the placement of the bodies at refrigeration temperature in the morgue. The immediate exposure of the dog aortas to carbon dioxide snow after their removal presumably prevented any significant change in the rela-

tive percentage values of the different riboflavin fractions.

The lack of a significant correlation (table 5) between the length of the period the bodies were kept refrigerated in the morgue and the percentage values of the riboflavin fractions in the human aortic samples indicates that little change in the riboflavin constituents occurred during that stage. Such storage, therefore, did not disqualify the samples for comparison between various age groups of observed riboflavin and riboflavin fraction values.

The observed decrease in the riboflavin content of the aortic tissue with age may be due to an actual decrease in the riboflavin concentration of the active aortic tissue, to partial displacement of normal structures by pathologic tissue, or to a combination of such changes. An evaluation of the relative significance of the suggested causes for the reduction in the riboflavin content of the samples with age is not possible at the present stage. It is of interest to point out in this connection that in the studies reported by Ferrebee and Weissman (3) a tendency to a decrease in the riboflavin content with age was likewise found in analyses of skeletal muscle, liver, heart, and kidney tissue. The number of observations in Ferrebee and Weissman's investigation is, however, too small to permit definite conclusions with regard to the effect of age on the riboflavin content of the organs.

SUMMARY

Determinations were made by the fluorometric method of Bessey, Lowry, and Love of the concentrations of free riboflavin + flavin mononucleotide, flavin adenine dinucleotide, and total riboflavin in 100 human aortic samples, derived from individuals ranging in age between 4 weeks and 83 years. Average values, respectively, of 0.40, 0.76, and 1.16 micrograms/Gm. fresh tissue were found. A tendency was noted for the riboflavin content of the tissue to decrease with the age of the subjects (coefficient of correlation Age/total riboflavin concentration: −0.39, t=4.20, N=100).

In analyses of 5 dog aortas mean values for free riboflavin, flavin mononucleotide, flavin adenine dinucleotide, and total riboflavin of, respectively, 0.14, 0.12, 2.31, and 2.57 micrograms/Gm. fresh tissue were observed.

In both human and dog aortic tissue the presence of an enzyme capable of splitting flavin adenine dinucleotide was demonstrated.

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STUDIES ON THE SUCCINIC DEHYDROGENASE OF HUMAN AORTIC TISSUE*

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The presence in human aortic tissue of a notable succinic dehydrogenase activity was demonstrated in a recent study from this laboratory (4), using a quantitative tetrazolium technique. In the present communication further experiments on the aortic succinic dehydrogenase will be reported. These studies include comparative observations on the respiratory intensity of the tissue in glucose-phosphate buffer and in succinate-phosphate buffer media; observations on the rate of tetrazolium reduction in glucose-phosphate and succinate-phosphate media; investigations on the inhibitory action of malonate on the tissue respiration and rate of formazan formation; and studies on the formation of l-malic acid by the aortic tissue in succinate medium.

The human aortas (descending thoracic section) employed in the investigations were obtained fresh at autopsy at the St. Louis City Morgue.† The preparation, under sterile conditions, of intima-media segments of the vessels was carried out as described previously (2).

I. Comparison of Q_{o_2} values of aortic tissue in glucose-phosphate buffer and succinate-phosphate buffer media.

For determination of the respiratory rate of the aortic tissue the procedure of Kirk, Hansen, Effersøe, and Iversen (3) was used. Two adjacent intima-media segments were employed for each experiment. One segment was suspended in a modified Krebs' phosphate buffer (2) with a glucose content of 0.2 per cent, and the other segment in equal volumes of the Krebs' buffer and 0.30 M sodium succinate solution. The pH of the media was 7.1. The Q_{o_2} values of the samples were determined in parallel 40-minute experiments at 37 C.

The results of experiments on 10 human aortas are presented in table 1. The observations show that the tissue respiration in succinate medium on the average was found to be 4.4 times higher than in glucose medium.

II. Comparison of rate of tetrazolium reduction by aortic tissue in glucose-phosphate buffer and succinate-phosphate buffer media.

In a recent study from this laboratory (4) an average reduction of triphenyl tetrazolium chloride of 0.013 mg./Gm. wet tissue/40 minutes was observed in experiments on aortic tissue suspended in glucose-phosphate medium, whereas under similar conditions a mean tetrazolium reduction of 0.107 mg. was found for aortic tissue maintained in succinate-phosphate medium. In the present investigation these observations were repeated by measurement of the formazan formation in glucose and succinate media by tissue samples obtained from adjacent parts of the same aortas as were employed for the oxygen consumption studies. The tetrazolium technique used was the same as that described in a previous publication (4).

The results of the tetrazolium reduction studies are likewise included in table 1. The data reveal a rate of formazan formation in the presence of succinate 4.0 times higher than in glucose medium.

III. Effect of malonate on aortic succinic dehydrogenase activity.

The inhibition of succinic dehydrogenase by malonate has been demonstrated by Quastel and Wooldridge (9), Quastel and Wheatley (8), and Krebs (5).

For investigation of the effect of malonate on the aortic succinic dehydrogenase two types of experiments were conducted, one in which the inhibition of the oxygen consumption of the tissue by various malonate concentrations was studied, and another in which the effect of malonate on the rate of tetrazolium reduction by the tissue was investigated.

The determinations of the effect of malonate on the tissue respiration were carried out in parallel experiments by the method of Kirk, Hansen, Effersøe, and Iversen as described

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TABLE 1. EFFECT OF SUCCINATE ON THE RESPIRATION AND TETRAZOLIUM REDUCTION BY HUMAN AORTIC TISSUE.

Sample No.	Sex	Age in Years	Q_{O_2}		Formazan Formation (Micrograms Formazan/Mg. Dry Tissue/Hour)	
			Glucose-Phosphate Medium	Succinate-Phosphate Medium	Glucose-Phosphate Medium	Succinate-Phosphate Medium
1.	F.	29	0.13	0.82	0.246	0.984
2.	M.	42	0.11	0.80	0.147	0.588
3.	M.	46	0.03	0.56	0.157	0.628
4.	M.	49	0.13	0.43	0.030	0.120
5.	F.	55	0.00	0.18	0.003	0.012
6.	M.	58	0.18	0.40	0.200	0.800
7.	M.	69	0.12	0.42	0.039	0.156
8.	M.	70	0.07	0.23	0.075	0.300
9.	F.	71	0.14	0.46	0.128	0.512
10.	M.	75	0.04	0.07	0.006	0.024
Mean			0.10	0.44	0.103	0.412

above. Intima-media segments of approximately 1.0 Gm. wet weight from the same aorta were suspended in succinate-buffer medium, and in succinate-buffer medium to which sodium malonate had been added, giving final malonate concentrations of 0.012, 0.025, and 0.050 M. Similar malonate concentrations were employed in the tetrazolium reduction studies.

The results of the experiments, presented in table 2, show a progressive inhibition of the succinic dehydrogenase activity with increasing malonate concentrations.

IV. Demonstration of l-malic acid formation by aortic tissue in succinate medium.

Succinic dehydrogenase catalyzes the oxidation of succinic acid to fumaric acid. Through the action of the enzyme fumarase the fumaric acid formed is hydrated to l-malic acid. Since it has been shown (6) that human aortic tissue possesses a comparatively high fumarase activity, the formation of l-malic acid from succinate was selected as a means of demonstrating the presence of succinic dehydrogenase in the aortic tissue.

TABLE 2. INHIBITION OF AORTIC SUCCINIC DEHYDROGENASE BY MALONATE.

Malonate Concentration of Medium	Q_{O_2}	Per Cent Inhibition	Formazan Formation Micrograms/Mg. Dry Tissue/Hour	Per Cent Inhibition
0.000 M	0.48	0	0.505	0
0.012 M	0.32	33	0.297	41
0.025 M	0.16	67	0.194	62
0.050 M	0.06	88	0.076	85

Experiments were conducted on 10 samples of human aortas. For each aorta 3 intima-media segments were used; the wet weight of the segments ranged between 0.9 and 1.1 Gm.

For each experiment two tubes (150 x 20 mm.) were prepared, containing 5.0 ml. of 0.05 M sodium succinate, pH 7.0, 4.25 ml. of 0.1 M phosphate buffer, pH 7.0, 0.005 ml. of 10 per cent calcium chloride solution, and 0.25 ml. of 0.1 N sodium arsenite solution.

The tubes were placed in a water bath at 39 C. for fifteen minutes for preheating of the substrate medium. At the end of this period an aortic segment was placed in each test tube, and aeration of the medium with oxygen started. After twenty minutes one of the tubes were removed from the water bath and rapidly chilled in ice water. The contents of the tube were then transferred to a pyrex grinder and the tissue homogenized. The homogenate was subsequently poured into a 25 ml. glass stoppered measuring cylinder containing 3.0 ml. of 50 per cent trichloroacetic acid. Rinse water from the homogenizer tube was added to the content of the cylinder. The volume was finally made up to 15.0 ml. with distilled water, and the contents mixed. At the end of sixty minutes the aortic sample in the other tube was treated in a similar way. Determinations of the l-malic acid content of the supernatants were carried out on 50 cu. mm. aliquots by the fluorometric method of Lowry and associates (7), as described in a previous publication (6).

The observed l-malic acid values were corrected by subtraction of a blank. The blank value was measured in each experiment by fluorometric determination of the supernatant obtained by trichloroacetic acid precipitation of a homogenate prepared from another segment of the aorta, which had not been subjected to incubation at 39 C. The blank provided a correction both for the small amounts of preformed malic acid in the tissue and for the fluorescence of the substrate medium and trichloroacetic acid solution used.

The results of the experiments are presented in table 3. The data show an average formation of 0.392 mg. l-malic acid/Gm. wet tissue in a 20-minute period, and a mean formation of 1.090 mg./Gm. wet tissue in a 60-minute period.

TABLE 3. FORMATION OF l- MALIC ACID BY HUMAN AORTIC TISSUE IN SUCCINATE-PHOSPHATE MEDIUM.

Sample No.	Sex	Age in Years	l- Malic Acid Formation	
			20-Minute Period Mg./Gm. Wet Tissue	60-Minute Period Mg./Gm. Wet Tissue
11	M.	43	0.110	0.390
12	M.	47	0.398	0.786
13	M.	47	0.380	0.850
14	M.	58	0.342	1.664
15	M.	62	0.652	1.324
16	F.	63	0.134	0.580
17	M.	65	0.202	0.640
18	M.	69	0.552	1.686
19	M.	72	0.446	1.436
20	M.	79	0.710	1.536
Mean			0.392	1.090

DISCUSSION

A review of the few reports in the literature dealing with the demonstration of succinic dehydrogenase in arterial tissue through the histologic tetrazolium technique was given in a previous publication (4) from this laboratory. In that communication the results of quantitative determinations of the rate of tetrazolium reduction by human aortic tissue in succinate medium and in various other media were also reported.

To the knowledge of the authors the only previous investigation on the oxygen consumption by arterial tissue in succinate medium is a study by Briggs, Chernick, and Chaikoff (1) on rat aortas, using the manometric Warburg technique. In experiments on intact aortas from young rats Briggs and his associates observed an average Q_{o_2} value in glucose-phosphate medium of 1.1, and in succinate-phosphate medium of 2.6. For aortas obtained from old rats the corresponding Q_{o_2} values were 1.1 and 2.3. In comparison with the data reported in the present study the effect of succinate on the respiration of rat aortic tissue would thus seem to be somewhat lower than in the case of human aortas.

SUMMARY

In experiments on intact human aortas the respiration of the tissue in succinate-phosphate medium was found to be 4.4 times higher than in glucose-phosphate medium, and the rate of tetrazolium reduction 4.0 times higher. Addition of malonate to the succinate medium caused a significant inhibition of both the oxygen consumption and formazan formation by the tissue.

A notable formation of l-malic acid was observed by aortic tissue maintained at 39 C. in succinate medium aerated with oxygen.

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THE UTILIZATION OF NUTRIENTS BY DOGS OF DIFFERENT AGES.*

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SEVERAL publications are available in the literature concerning attempts to determine if changes in the metabolism occur in connection with the aging of animals. A review of such publications is given in the third edition of *Cowdry's Problems of Ageing* (2).

Bogdonoff, Shock, and Nichols (1) concluded recently that the magnitude of loss of calcium was no different for old and young men when both were fed diets relatively low in this element. Liu and McCay (4) found that the ability of pups to retain calcium when the dietary intake was low was inversely related to the age of the pups. Old dogs were found to have substantial difficulties in the maintenance of calcium equilibrium. Hansard and associates (3) found that calcium absorption was greatest in young cattle, decreased rapidly to sexual maturity, and again declined in older animals.

In the course of testing the chromic oxide method for chemical balances, Lloyd and McCay (5) found that old dogs were somewhat superior to young ones in their ability to utilize the protein and fat of a common dog meal.

The current study was designed to determine the effect of age in the utilization of dry matter, crude protein, fat carbohydrate, nitrogen, calcium, and phosphorus in a series of food mixtures differing widely in composition.

EXPERIMENTAL PROCEDURE

(a) Animals. Twelve female Beagles were maintained in individual metabolism cages throughout the experiment. The animals were selected so that 3 distinct age groups were available for comparison. The first age group was composed of 4 dogs showing definite signs of aging, i.e., decreased activity and appetite, greying hair coat, etc. These old dogs ranged in age from 9 to 12 years, with an average of 10½ years. The second age group consisted of 4 fully matured, healthy and active dogs, all approaching 2 years of age at the beginning of the test. The final age group consisted of 4 pups, all 3 months of age at the beginning of the trial.

(b) Diets. Six diets, compounded to provide different combination of protein and fat levels, were fed to all dogs. A description of these diets is given in table 1.

When grouped by protein level, 2 high protein (E and G), 2 medium protein (C and D), and 2 low protein (F and H) diets were fed. When grouped by fat level, 3 high fat (D, G, and H) and 3 low fat (C, E, and F) diets were fed. It should be noted that within each of the 3 protein levels, a high and a low fat diet was included, and within each of the 2 fat levels, a high, medium, and low protein diet was included. This design provided for a comparison between age groups of the 3 levels of protein, of the 2 levels of fat and of diets ranging from the high protein, high fat diet, through all combinations, to the low protein, low fat diet.

The 6 diets were fed in the order in which they appear in table 1. The old dogs and the young adults were fed once daily, while the pups were fed twice daily. The diet allowance for each dog was fixed as the maximum amount that the animal would consume rapidly and completely at a feeding.

(c) Balance Trials. A balance trial was carried out for each dog for each diet fed. The Cr_2O_3 method of determining nutrient output in the feces, tested for dogs by Lloyd and McCay (5), was employed throughout the experiment. Chromic oxide was included in each diet to the extent of 1 per cent of its dry matter content. A preliminary adjustment period of 10 days was followed by a period of 4 days during which fecal samples were collected and total urinary output was recorded for each dog. The daily fecal samples were dried at 100 C°, composited for the 4-day period, and ground for analysis.

One-tenth of the daily urinary excretion was refrigerated under toluene, and at the con-

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TABLE 1. COMPOSITION OF 6 DIETS FED TO 3 AGE GROUPS OF DOGS.

Diet	Physical Composition of Diets	Relevant Chemical Composition				
		Crude Protein (%)	Fat (%)	Total Carbohydrate (%)	Calcium Mg./Gm.	Phosphorus Mg./Gm.
C	Dog meal* (M.)	27.4	8.1	55.8	19.5	13.0
D	M. (72.5%) + lard (27.5%)	19.9	33.4	40.5	14.1	9.4
E	M. (76.4%) + casein (23.6%)	40.5	6.3	45.8	15.8	11.0
F	Cornflakes	9.7	5.6	81.0	1.3	7.2
G	M. (18.0%) + casein (44.0%) + lard (38.0%)	41.5	39.6	16.0	5.3	4.5
H	Cornflakes (64.0%) + lard (36.0%)	6.2	39.6	51.8	0.8	4.6

*The open formula dog feed had the following composition: alfalfa meal 1.5 (%), meat scrap 18, fish meal 1.5, soybean meal 8.5, tomato pomace 2.0, corn flakes, P.C. 20.3, wheat flakes 5.0, fat-flour biscuit 25.0, wheat germ 8.5, milk 4.0, cheese meal 1.5, salt .20, dicalcium .75, Brewer's yeast 2.0, irradiated yeast .10, cod liver oil .075, liver meal 1.0, chlorophyllin .075.

clusion of the 4-day period, the composited aliquots were analyzed.

(d) Chemical Analyses and Balance Data. Each composite fecal sample was analyzed for total ash, crude protein (nitrogen x 6.25), fat (as ether extract), calcium, and phosphorus by standard procedures. Cr_2O_3 was determined by the method of Schurch and associates (6). Because of the extremely low crude fiber value of all diets, total carbohydrate was determined by difference. The composited urine aliquots were similarly analyzed for nitrogen, calcium, and phosphorus.

These analyses enabled a determination of 1) the apparent digestibility of dry matter, protein, fat, and total carbohydrate, and 2) of the nitrogen, calcium, and phosphorus balance for each dog for each diet fed.

RESULTS

The average apparent digestion coefficients of dry matter, crude protein, fat and total carbohydrate, for each of the diets by each age group of dogs, are shown in table 2.

It is obvious from these results that no one age group consistently digested the nutrients of the different diets to a greater or lesser extent than did either of the other age groups. For example, the dry matter of diet C was digested to a greater extent by the old dogs and pups than by the young adults, that of diet E was better digested by the pups than by the old dogs or young adults, while that of diet H was better digested by the old dogs and young adults than by the pups. Taking the mean digestibility of dry matter for the 6 diets, it may be noted that the old dogs digested this complex fraction to a significantly greater extent than did either the young adults or pups.

The over-all picture of total carbohydrate utilization showed that the old dogs and pups were superior to the young adults in their ability to digest this fraction of the diet. As in the case of dry matter, inconsistencies were apparent between diets. For example, the carbohydrate of diet C was digested to a greater extent by the old dogs than by the young adults or pups, while that of diets E and G was better digested by the pups than by the old dogs or young adults.

Of particular interest is the fact that the mean apparent digestibility value for both the crude protein and the fat in the 6 diets was significantly higher for the old dogs and young adults than for the pups. In the case of crude protein, this was a reflection of a much decreased apparent digestibility of the fraction by the pups where the initial level of protein in the diet was low (diets F and H). In the case of fat, the immature animals were obviously less well equipped to

TABLE 2. AVERAGE APPARENT DIGESTIBILITY OF THE NUTRIENTS OF 6 DIETS BY 3 AGE GROUPS OF DOGS.

Nutrient	Age Group	Average Apparent Digestion Coefficients*						Necessary Difference (P = 0.05) Between Age Means	Mean of Diets	Necessary Difference (P = 0.05) Between Diet Means
		Diet C (%)	Diet D (%)	Diet E (%)	Diet F (%)	Diet G (%)	Diet H (%)			
Dry matter	Old dogs	75	82	78	73	92	83		80	
	Young adults	72	80	78	72	92	82	1.7	79	0.7
	Pups	74	80	81	72	93	77		79	
Crude protein	Old dogs	76	76	87	55	94	54		74	
	Young adults	72	72	86	61	95	57	3.4	74	1.4
	Pups	73	75	85	45	94	43		69	
Fat	Old dogs	92	98	93	81	99	98		93	
	Young adults	88	97	91	80	99	97	4.2	92	1.7
	Pups	88	92	95	82	97	82		89	
Total carbohydrate	Old dogs	79	79	77	76	78	77		78	
	Young adults	77	78	77	75	78	76	1.9	77	0.8
	Pups	77	77	79	76	81	78		78	

*Each value represents the average of 4 dogs.

handle high fat diets (principally diets D and H) than were the 2 mature groups of dogs. This situation was emphasized by the frequent occurrence of diarrhea in the pups fed diets containing excessive amounts of fat.

These results with protein and fat indicated the desirability of grouping the 6 different diets according to either their crude protein or their fat levels. In order to conserve space, the regrouped data have not been included.

When the diets were grouped by level, it was found that age had no effect on the apparent digestibility of either the crude protein or the fat in the high protein diets. The significantly increased apparent digestibility of dry matter in these diets by the pups was a reflection of an increased utilization of carbohydrate and of mineral material. On the other hand, the old dogs digested to a significantly greater extent the dry matter and the crude protein in the medium protein diets than did the young adults, with the pups holding an intermediary position with respect to these nutrients. The old dogs and young adults were superior to the pups, and the old dogs were superior to the young adults and pups in their ability to digest the fat and carbohydrate, respectively, in the medium protein diets. In the case of the low protein diets, dry matter was better digested by the old dogs and young adults than by the pups, this being partially due to an increased apparent digestibility of both crude protein and fat by the former age groups. Of particular interest was the low value for pups for the apparent digestibility of crude protein in the low protein diets. It should also be noted that the young adults digested the low level of protein to a significantly greater extent than either the very young or very old dogs.

The most significant finding arising from the grouping of the diets by fat level was the decreased ability of the pups to digest large amounts of fat as compared to the old dogs and young adults. The fat in the low fat diets was equally well digested by the old dogs and pups, but the young adults were inferior to the old dogs in their ability to utilize these smaller amounts of fat. The crude protein in both the high and low fat diets was better digested by either group of mature animals, while total carbohydrate was digested to the same

TABLE 3. AVERAGE NITROGEN, CALCIUM, AND PHOSPHORUS INTAKE AND BALANCE* ON 6 DIETS BY THE 3 AGE GROUPS OF DOGS.

Nutrient	Age Group	Diet C		Diet D		Diet E		Diet F		Diet G		Diet H		Necessary Difference (P=0.05) Between Age Means	Mean of Diets	Necessary Difference (P=0.05) Between Diet Means
		Intake Gm./Day	Balance Gm./Day	Intake Gm./Day	Balance Gm./Day	Intake Gm./Day	Balance Gm./Day	Intake Gm./Day	Balance Gm./Day	Intake Gm./Day	Balance Gm./Day	Intake Gm./Day	Balance Gm./Day			
Nitrogen	Old dogs	9.13	+0.68	6.59	+0.76	12.65	+0.98	2.68	−0.69	11.89	+2.04	1.84	−0.77	0.95	+0.50	0.39
	Young adults	9.13	+0.62	6.59	+0.49	13.39	+0.05	3.21	−0.31	13.47	+1.68	1.89	−0.62		+0.32	
	Pups	6.08	+1.41	4.40	+1.09	8.93	+2.37	2.28	−0.14	10.78	+2.43	1.48	−0.27		+1.15	
Calcium	Old dogs	4.04	+0.17	3.00	+0.04	3.08	−0.29	0.23	−0.27	0.94	−0.16	0.16	−0.18	0.11	−0.11	0.05
	Young adults	4.04	−0.01	3.00	−0.08	3.26	−0.22	0.27	−0.20	1.07	−0.05	0.16	−0.19		−0.12	
	Pups	2.70	+0.70	2.00	+0.56	2.18	+0.72	0.19	−0.09	0.85	+0.53	0.12	−0.10		+0.39	
Phosphorus	Old dogs	2.70	+0.34	2.00	+0.22	2.15	−0.20	1.24	+0.27	0.80	+0.12	0.85	+0.16	0.13	+0.11	0.05
	Young adults	2.70	+0.22	2.00	+0.26	2.28	−0.03	1.49	+0.49	0.90	−0.08	0.88	+0.23		+0.12	
	Pups	1.80	+0.54	1.33	+0.42	1.52	+0.37	1.06	+0.34	0.72	+0.39	0.69	+0.31		+0.39	

*Each value represents the average of 4 dogs.

extent by all age groups regardless of fat level grouping.

In the over-all picture it is evident that all ages make excellent use of the protein of casein and rather poor use of the protein of corn flakes. Confirming earlier observations made upon dogs by unknown age and breed it is evident that fat in the form of lard is quite well utilized by all ages of beagles.

The average nitrogen, calcium, and phosphorus balance data of the 6 diets by the 3 age groups of dogs are given in table 3.

Although nitrogen retention in all diets was higher for the pups than the 2 mature age groups, only in the case of the high protein, low fat diet (E) was the difference statistically significant. Except for the low protein diets (F and H), the retention of nitrogen was greater by the old dogs than by the young adults; however, in no case were the differences between these age groups of significance.

Except for the low protein, high fat diet (H), where there was no difference between age groups, the pups exhibited the greatest ability to retain the ingested calcium. In addition, the retention of calcium was significantly higher by the old dogs than by the young adults in the case of the 2 medium protein diets (C and D), while this situation was reversed for the high protein, low fat diet (G). No difference in calcium balance between the mature age groups was found for diets E and F.

With the exception of the low protein, low fat diet (F) where there was no difference between age groups, the greatest retention of ingested phosphorus was displayed by the pups as well. There was no significant difference in phosphorus balance between the old dogs and the young adults for any of the diets.

When nitrogen, calcium, and phosphorus balance data were averaged for the 6 diets, there was no real difference between the old dogs and young adults, but both groups were inferior to the pups in their ability to retain any of these nutrients.

During periods of stress in regard to the lack of calcium and suitable protein as occurred during the feeding of diets F and H, the puppy is able to retain its supply of the above nutrients more effectively than the adult.

Regardless of protein level in the diet there was no significant difference in nitrogen balance between the old dogs and young adults. However, the pups had a significantly higher nitrogen balance when consuming the high protein diets than either of the mature age groups, while for the medium protein diets, the pups were superior to the young adults, but essentially similar to the old dogs in their ability to retain the ingested nitrogen. No differences in nitrogen balance were observed between age groups in the case of the low protein diets.

The calcium and phosphorus levels of the diets were not controlled in any way. However, upon observation it was found that they could be grouped arbitrarily into high and low calcium or high, medium and low phosphorus diets.

Regardless of dietary level of calcium, the old dogs and the young adults were alike in their ability to retain calcium, and both were inferior to the growing animals in this respect.

As with calcium, there was no significant difference between the 2 mature age groups in phosphorus balance. In the case of the high and low phosphorus diets, the pups had a significantly higher retention of phosphorus than either the old dogs or young adults, while for the medium phosphorus diets only the difference between the pups and old dogs was significant.

DISCUSSION

In a preceding study (5) using a dog meal of the same composition as that used in this study, it was found that the crude protein and ether extract of the meal were better utilized by old than by young dogs. This study with different dogs of the same breed confirms the previous observations.

When considering the mean apparent digestion coefficients of the nutrients of the 6 diets fed in this experiment, it was noted that advanced age was not a factor in reducing the apparent digestibility of any nutrient. In fact, in the case of dry matter, the old animals exhibited a greater digestibility than did either of the younger age groups. The signifiance of these findings is emphasized when consideration is taken of the evidence in the literature that indicates various metabolic defects in old age which gives rise to the as-

sumption that the digestive ability of the aged may also be impaired.

Growing animals failed to digest either crude protein or fat to as great an extent as the mature age groups. When diets were grouped by protein level, it was found that the low over-all digestibility of crude protein by the pups was due wholly to a reduced ability to digest that nutrient in the low protein diets. However, because the protein in those diets originated entirely from corn, it was impossible to determine from this trial whether low quality or quantity of protein was responsible for this decreased utilization. When the diets were grouped by fat level, the low over-all digestibility of fat by the pups was shown to be due to a reduced ability to digest large amounts of fat.

The mean values for the nitrogen, calcium, and phosphorus balance of all diets by the 3 age groups of dogs gave expected results in so far as the growing animals were concerned. That is, a greater retention of each of these nutrients was shown for the pups than for either of the mature age groups. However, no diminished retention of nitrogen, calcium, and phosphorus by the aged animals when compared to the young adults was observed. When the diets were grouped by protein, calcium, or phosphorus level, there was still no difference in nitrogen, calcium, or phosphorus balance, respectively, between the mature age groups. These latter observations are contrary to currently accepted concepts of the effect of advancing age on the retention of nitrogen, calcium, and phosphorus. Possibly, the oldest groups were not old enough.

These results with calcium may seem contrary to the earlier findings of Liu and McCay (4); however, Liu compared only dogs that were still growing at 34 weeks of age with old adults, while this study included young adults that were through growing at 2 years of age.

SUMMARY

1. Advanced age in dogs did not reduce the apparent digestibility of dry matter, crude protein, fat, or total carbohydrate when the mean digestion coefficients of these nutrients for 6 different diets were considered.

2. When compared to young adult or old dogs, pups were less able to digest the crude protein in low protein diets. Whether this reduced apparent digestibility was due to the low quality or low quantity of the protein is not known at this time.

3. Pups were inferior to young adults or old dogs in their ability to digest large amounts of fat.

4. During periods of deficiency in protein or calcium, as when these nutrients are provided at a very low level by corn flakes, the pups have much greater power of conserving their body stores than have the older dogs.

5. The protein of corn flakes is very poorly utilized by dogs of all ages while that of casein is well utilized. Lard is a form of fat well utilized by beagles of all ages.

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BOOK REVIEWS

GERIATRIC MEDICINE, Medical Care of Later Maturity, 3rd edition, edited by Edward J. Stieglitz, J. B. Lippincott Company, Philadelphia, 1954, 718 pages, $15.00.

Geriatric Medicine—medical care of later maturity—edited by Dr. E. J. Stieglitz and written by him and a large number of other contributors is outstandingly the greatest contribution to this field of medicine.

It is written essentially for the medical profession and should be read by every physician. It should be used as a textbook for medical students and no medical library should be without a copy for reference. The third edition is a worthy successor to the previous editions, is well arranged, and has a good index.

Section I—basic considerations—is an excellent introduction to the subject of geriatric medicine. It is interesting to read and contains most useful information concerning the anatomy, physiology, and psychology of later maturity. It is obvious that the contributors have not only a sound philosophy towards and a sympathetic understanding of older persons, both in health and in sickness—but it is evident that they have also a very real approach to and a practical experience of the problems likely to occur.

The sub-sections include such interesting and important subjects as intellectual changes, mental hygiene, medico-legal aspects, essentials of geriatric surgery, anaesthesia, and nursing. In a work of this size one could hardly expect a uniform standard, but as "essentials of nursing" was included it is disappointing that no practical advice is given.

The remaining sections follow the more orthodox medical textbooks, but in every case attention is drawn to the special points associated with older patients and points of deviation are explained. Treatment is given precisely and in detail, which makes the book useful for reference. One regrettable omission is the scant reference to urinary incontinence, which is so common an accompaniment to old age.

The whole book is richly illustrated by diagrams, tables, and x-ray reproductions, which add to its value and usefulness.

In spite of a few omissions it is a book which no doctor should fail to get, to use and to keep.

It is a pleasure to write a review of a book, which can honestly be an appreciation.

MARJORY W. WARREN
Consultant Physician
London, England

LES ABEILLES, Source de Jouvence et de Vitalité, Le Pollen et la Gelée Royale, Propriétés, Récolte, Usages, by Alin Caillas. Chez L'Auteur, 40, Boulevard Aristide-Briand, Orléans (Loiret), France, 96 pages, 1953.

Caillas, Ingénieur agricole Lauréat de l'Académie d'Agriculture, has written one of the few existing books on bee pollen and royal jelly. Professeur E. Roubaud, Vice-President de la Société Centrale d'Apiculture, Membre de l'Institut et de l'Académie d'Agriculture, has written the Preface for this little book.

The identification of two longevity factors, pantothenic acid and pyridoxin, in royal jelly (Gardner, *J. Gerontol.*, *3:* 1-8; 9-13, 1948) has stimulated interest in this material. Unfortunately interest has not been stimulated for further scientific research, as the only follow-up investigation has been Fritsch's work on Daphnia (*Ztschr. f. Wissen. Zoöl., 157:* 35-56, 1953) in which a two-fold increase in life span was obtained by rearing the organisms in a medium containing 0.2 Gm. pantothenic acid per liter.

However, popular and commercial interest in royal jelly has become practically a fad. (*Point de Vue Images du Monde*, Feb. 5 and 12, 1953). At the present time royal jelly is being eaten, injected subcutaneously into people, and even has been included in face creams for skin "rejuvenation" in both Europe and America. Fantastic claims made for the youth promoting qualities of royal jelly have appeared in popular articles but supporting clinical work has not appeared to the reviewer's knowledge.

Due to this wide-spread interest in bee pollen and royal jelly Caillas' book is a timely publication whether the claims are ever verified or not. The book is fascinating to read and about two-thirds of it is devoted to the very practical and useful techniques of increasing the yield of royal jelly in hives, to collecting pollen and royal jelly, and finally preservation and reported uses of the material. The practical methods of bee keeping described are well illustrated and thus are of benefit to the apiculturists.

The remainder of the book consists of optimistic statements regarding the therapeutic use of pollen and royal jelly. In the reviewer's opinion whatever chemical components of royal jelly that have therapeutic properties, both for general health and for longevity purposes, should be carefully investigated in rats using the pure synthetic materials in order to control the dosage exactly before human use.

Caillas' book is also interesting to the gerontologist as he briefly includes descriptions of other attempts at rejuvenation and treatments for the aging syndrome. He is very enthusiastic about royal jelly and describes several "cases" using it and their reported benefits.

This book is a warning to the gerontologist in that unless prophylactic treatments are developed for the aging syndrome, then the populace will use empirical treatments, sometimes at great cost, and irrespective of the clinical efficacy.

The book is marred by a very poor reference system so that only one familiar with the fields of gerontology, nutrition, and apiculture in both Europe and America can use it with any degree of confidence.

T. S. GARDNER
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BOOKS RECEIVED

Books received since January 1 are acknowledged in the following list.

Les Abeilles, Source de Jouvence et de Vitalité, le Pollen et la Gelée Royale, Propriétés, Récolte, Usages, by Alin Caillas. Chez l'Auteur, 40, Boulevard Aristide-Briand, Orléans (Loiret), France, 1953, 96 pages, (reviewed in this issue, page 188).

Peripheral Vascular Diseases, by Edgar V. Allen, Nelson W. Barker, and Edgar A. Hines, Jr., W. B. Saunders Company, Philadelphia, 1955, 825 pages, $13.00.

Standard Values in Nutrition and Metabolism, edited by Errett C. Albritton, W. B. Saunders Company, Philadelphia, 1954, 380 pages, $6.50.

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Section B

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SEX DIFFERENCES AND EDUCATIONAL INFLUENCES ON A MENTAL DETERIORATION SCALE

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WITH THE increasing attention now being placed on our aging population, the problem of the relative influences of heredity and environment becomes of real importance in the process of aging. One aspect of this area is the question of sex differences in mental deterioration. Considerable work has been directed toward possible differences in intelligence, but no consistent pattern has been found (3, 4, 6, 7, 8, 11, 15, 20). Such diversity of findings is undoubtedly due, in part at least, to different measuring instruments and diverse samples employed. As far as mental deterioration is concerned very little has been reported that is directed to the possibilities of sex differences in deterioration.

In a like manner many studies have been conducted to demonstrate the effect of education on intelligence, but little attention has been given to the influence of education on mental deterioration. Perhaps this is due to the opinion held by many that mental deterioration scores and intelligence scores are closely related.

There have been a number of tests of mental deterioration constructed on a differential test score rationale (9, 17, 18, 19). The basic assumption here is that some psychologic functions have been noted to show very little if any decline with advancing age, while others show a rather marked decline. By equating these various functions at the presumable peak of the majority of people's life, it becomes possible thereafter to use those tests which show little decline with age as an estimate of the psychologic functions at the individual's prime. Thus in effect, it is argued, there is at least an adequate substitute for the more desirable longitudinal test-retest method.

Perhaps the best known scale of this type is the use of various subtests of the Wechsler-Bellevue Intelligence Scale for adults. Originally Wechsler (19) proposed the use of Information, Vocabulary, Picture Completion, and Object Assembly as the "hold" tests and Digit Span, Arithmetic, Block Design, and Digit Symbol as the "don't hold" tests. The assumption here, besides having tests that meet the requirements as outlined above, was to have an equal number of tests from the verbal and performance scales in each of the "hold" and "don't hold" batteries. Other investigators (1, 10) have suggested the use of different subtests, however.

The purpose of this study was to examine the possibility of sex differences in deterioration and the effect of education on mental deterioration as measured by a scale composed of some of the subtests of the Wechsler-Bellevue Intelligence Scale.

METHOD

Six subtests of the Wechsler-Bellevue Intelligence Scale Form I were administered to 50 American born men (60 to 89 years, mean 72.1) and 50 women (60 to 84 years, mean 71.1). All of the subjects were considered free from any physical or emotional handicaps that could hinder their performance on the tests. Table 1 indicates the number of subjects in each 5-year age group. Considerable difficulty was encountered in attempting to equate the two groups for age. After those individuals who had visual, auditory, or motor handicaps, those who were foreign born, and those who for one reason or another were unwilling to take the tests, were eliminated from the group of possible subjects extensive equating was virtually impossible. Nevertheless, the t ratio of the difference in age between the men and women was only .65, which indicates a fairly good control of this variable.

The subjects were drawn from the urban and suburban areas of Salt Lake City and from private and charitable homes for the aged in this area. Table 2 indicates the number and location from which the subjects were drawn.

The educational achievements of the subjects are indicated in table 3. If an individual had attended more than a half-year of a given level of schooling he was put in that category.

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TABLE 1. NUMBER OF SUBJECTS IN EACH 5-YEAR AGE GROUP.

Age	Men	Women	Both Sexes
60–64	10	10	20
65–69	11	12	23
70–74	10	11	21
75–79	10	6	16
80–84	5	11	16
85–89	4	0	4
	N = 50	N = 50	N = 100

TABLE 2. RESIDENCE OF SUBJECTS.

Sex	Homes for Aged	Community
Men	20	30
Women	18	32
Both sexes	38	62

TABLE 3. EDUCATIONAL LEVELS OF SUBJECTS. (Mean Age of Each Group in Parentheses)

Sex	College	High School	Grade School
Men	15 (70.1)	15 (75.0)	20 (71.2)
Women	10 (71.2)	17 (71.5)	23 (71.1)
Both sexes	25 (70.5)	32 (73.1)	43 (71.1)

TABLE 4. MEAN DETERIORATION AND INTELLECTUAL FUNCTIONING OF THE MEN AND WOMEN.

	Men	Women	t Ratios of Differences
Deterioration	.48	.44	1.43
Verbal I.Q.	120.2	114.6	2.60*
Performance I.Q.	105.6	104.8	1.40
Full Scale I.Q.	112.5	108.9	1.58

*Significant at .05 level

University, business college, music school, or any recognized formal education beyond high school was considered as college education. The greatest variability of the mean ages of the educational groups was 4.9 years and in no instance did the t ratios reach the .05 level of significance (see table 6).

The 6 subtests administered to the group were Information, Comprehension, and Vocabulary comprising the "hold" tests and Block Design, Picture Arrangement, and Digit Symbol comprising the "don't hold" tests. It should be noted that these are not the same tests as suggested by Wechsler (19). A number of investigators (2, 5, 10, 14, 16) have found that the subtests Information, Vocabulary, and Comprehension consistently prove to be the most resistant to advancing age, while Picture Arrangement, Block Design, and Digit Symbol were the most vulnerable. It seemed that the use of these 6 subtests as a deterioration scale would be the most sensitive measure of mental deterioration that one could find in using the Wechsler-Bellevue subtests. It should be noted that the "hold" tests are all verbal tests while the "don't hold" tests are performance tests. While this does not meet the rationale of Wechsler's choice of tests, it seemed desirable to maximize the deterioration scores for this study.

In computing each individual's mental deterioration score, the "don't hold" tests were totalled and subtracted from the sum of the scores of the "hold" tests. The difference was then divided into the "hold" tests score. Thus a deterioration score of 30 per cent indicates that the individual has declined 30 per cent from his original level in those functions that are measured by the "don't hold" tests.

By estimating each individual's verbal, performance, and full scale intellectual functioning from the tests used it was possible to determine the relationship between intelligence and mental deterioration.

RESULTS AND DISCUSSION

The mean deterioration, verbal, performance, full scale intelligence, and respective t ratios are presented in table 4. It should be noted that in only one instance did a t ratio reach the .05 level of significance. This was in verbal intelligence, a difference in favor of the males. The rather large difference between the verbal and performance scores on

the combined groups is probably best accounted for in that the estimated 5-test score was determined from those 3 tests which hold up best in the verbal scale and those 3 that decline most in the performance scale. The women showed slightly less deterioration than the men. This difference was not statistically significant, however.

Subjectively there was little difference between the two sexes in their approach to the problems. More apparent than any sex differences were the marked differences of the group as a whole when compared with the typical behavior of a younger person in taking the tests. The older subjects showed definite impairment in their ability to solve the Picture Arrangement and Block Design tests. With the Picture Arrangement test attempted solutions were apparently by trial and error, or at least each picture was dealt with individually, rather than as part of a meaningful unit.

On the Block Design test the subjects very often decided on a possible method of constructing the design, and then proceeded without any further reference to the design model until they were through. As would be expected, there was often marked differences between the model and the construction. There was very little individual scatter on the Picture Arrangement and Block Design tests. Once an individual missed one of the stories or designs, it was unusual for him to complete correctly a subsequent problem in that test. This is in contradiction to Lorge's (12, 13) hypothesis that the main impairment of the senescent is his inability to react rapidly. In the present study, if the subject did not solve the problem within the allotted time, the probability was that he would be unable to solve it within any reasonable length of time. Lorge's hypothesis was fairly well borne out, however, in the Digit Symbol test. It would seem that the slower rate of responding resulted from both their inability to write rapidly and to shift their trend of thought rapidly.

Educational Influences

The performance of the senescents (both sexes combined), classified according to their level of school is indicated by tables 5 and 6. Of primary importance here is the indication that deterioration is evidently independent of the person's level of schooling. In no instances were there any t ratios that reached the .05 level of significance so far as deterioration was concerned. This would seem to argue for a maturational point of view at least so far as this deterioration index is concerned.

TABLE 5. MEAN DETERIORATION AND INTELLIGENCE CLASSIFIED ACCORDING TO EDUCATIONAL LEVEL OF BOTH SEXES COMBINED.

	College	High School	Grade School
Deterioration.....	.42	.48	.47
Verbal I.Q.....	124.7	118.8	112.6
Performance I.Q..	111.4	105.7	101.3
Full Scale I.Q.....	118.4	110.9	105.7

TABLE 6. t RATIOS OF DETERIORATION, INTELLIGENCE, AND AGE OF BOTH SEXES COMBINED CLASSIFIED ACCORDING TO EDUCATIONAL LEVEL.

	Deterioration	Verbal I.Q.	Performance I.Q.	Total I.Q.	Age
College vs. High School....	1.26	2.32*	2.06*	2.48*	1.22
College vs. Grade School...	1.29	4.62†	4.04†	4.67†	.31
High School vs. Grade School...	.17	2.33*	2.16*	2.16*	1.04

*Significant at .05 level
†Significant at .01 level

As one would expect, significant differences were indicated consistently between the groups in intellectual functioning. This coupled with the finding above is considered by the writer to suggest the independence of the deterioration index from intellectual functioning. It would seem that this measure of deterioration has considerable merit from this

point of view. To measure mental decline effectively it is desirable to have an index which is equally applicable to all levels of ability. It would seem probable that the same generalizations would likewise apply to Wechsler's deterioration index.

It should be emphasized that this study was not concerned with the potentiality of the deterioration index as a measure of brain damage. The only interest here has been with the subjects who were quite normal behaviorally.

SUMMARY

Fifty normal men and 50 normal women between the ages of 60 and 89 were administered 6 subtests of the Wechsler-Bellevue Intelligence Scale Form I in an effort to determine the possibilities of sex difference in mental deterioration and the effect of education on mental deterioration.

In treating this latter problem the total group was divided into 3 categories: Those who had attended college, those with high school education, and those with grade school education. Verbal, performance, and full scale intelligence scores were estimated and the differences in deterioration and intelligence were considered statistically. It was concluded that:

1. There is no significant difference in deterioration between men and women.
2. The deterioration index is independent of educational influences.
3. The deterioration index is independent of the level of intelligence.

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PERSONALITY CHANGES WITH AGE: AN ITEM ANALYSIS OF THE MINNESOTA MULTIPHASIC PERSONALITY INVENTORY*

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THE PRESENT paper deals with one facet of an interdisciplinary study on aging. The over-all program places emphasis on the aging of the cardiovascular system. It combines the cross-sectional and longitudinal approach, and is focussed on the changes during maturity (adulthood) rather than the old age (senility).

The subjects report once a year for a thorough medical examination, a battery of biochemical, physiologic, and psychologic tests, and anthropometric measurements. In the personality area the Minnesota Multiphasic Personality Inventory (abbreviated as MMPI), a special Cardiovascular Inventory, Rosenzweig's Picture-Frustration Study, a shortened Thematic Apperception Test, and Cattell's 16 P(ersonality) F(actor) Questionnaire have been administered since the inception of the study in 1947. The MMPI was used both as a standardized inventory, evaluated with reference to the established clinical scales, and as a pool of relevant items descriptive of variations in several areas or aspects of personality. Results of an item analysis of the Cardiovascular Inventory, covering in part the same facets of personality and yielding a similar but less comprehensive picture of age differences between young and middle-aged normal men, were presented in a separate communication (1).

METHOD

The construction of the MMPI has been described in detail (13). The inventory was developed as means for characterizing personality in the frame of reference of clinical syndromes such as hypochondriasis, depression, and hysteria. There are several "validating" scales and a scale for measuring the masculinity-femininity of interests. The inventory consists of a large reservoir of items out of which the clinical scales were developed by contrasting normal individuals with patients exhibiting a particular clinical syndrome. In time additional scales were developed, such as the scale for measuring social introversion-extroversion (9) and a scale attempting to evaluate and take into account the effect of subject's attitudes toward the test situation (19).

In clinical practice a form of the inventory is frequently used in which each question is printed on a separate card and only a portion of the answers is recorded (and scored). For our purposes we wanted to have a permanent record of answers to all the items and, accordingly, presented the questions in a booklet form with the answers (Yes, Cannot say, No) recorded directly in the booklet. Because of the possibility of errors, particularly with the older age group, we did not use the commercially available group form in which the answers are made on a separate sheet. Due to extraneous factors, the first half of the younger group was given the inventory with the sequence of items identical with that in the Manual (14). For the second half of the younger men and for all older men the sequence of items in the group form was followed. Only the latter material was analyzed in detail in the present study.

SUBJECTS

Only men were used as volunteer subjects. They fell into two age categories, 17 to 25 (157 college students, mean age 20 years) and 45 to 55 (233 business and professional men, mean age 49 years). They had a normal blood pressure (less than 140 mm. Hg systolic and 90 mm. Hg diastolic) and were clinically in good mental and physical health. The two age groups were roughly comparable in national and social background.

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RESULTS: SCALE SCORES

In addition to the three validating scales ("?", L, and F) the MMPI was scored for Hypochondriasis and Introversion-Extroversion. The "?" scores, representing the number of items which were placed by the subject in the "Cannot say" category, were low throughout. The subjects were encouraged to give definite answers and they followed closely these instructions. On all the three validating scales 50 is the lowest "transformed" score. It was also the modal value, in both groups, which resulted in markedly skewed distributions. The mean value of the L score ("L" standing for "Lie," with a high score suggesting a very "good" opinion of the subject about himself and a tendency to portray oneself in a "socially acceptable light") was 51.3 in the younger group and 50.5 in the group of older men. The F scale has been designed to pick up such factors, invalidating the scores obtained on the clinical scales, as clerical errors involved in recording the results of the card-form of the inventory, inability of the subject to give relevant answers, or a careless way of responding to the items. The average scores were also very low (51.6 in both groups), indicating high internal validity of the inventory for the given subjects. On general grounds such results could be well expected because the subjects were intelligent and fully cooperative.

The mean transformed scores on the Hypochondriasis and Introversion-Extroversion scales together with standard deviations of the scores, the differences between the means, and the respective t-values are given in table 1. The younger men have somewhat lower mean scores on both scales but the difference reaches a 1 per cent level of statistical significance only for the Introversion-Extroversion scale. The older men are somewhat more "introverted," a tendency noted in older people also by Gray (12). In reference to norms for the general adult population—with a mean transformed score of 50 and a standard deviation of 10—both groups have somewhat lower mean scores (and standard deviations) on both scales. However, the differences are small and the older men, at any rate, can be regarded as a sample of the normal adult population as far as these two characteristics are concerned. The mean value of the Hypochondriasis score in the younger men (47.6) is very close to the weighted mean of 47.3 obtained from data on 1295 college students brought together by Brown (3).

Scoring the inventory for all the available clinical scales did not seem particularly rewarding at this time. The average profile of the older men, except perhaps for some deviation on the Masculinity-Femininity scale and on Hypomania, would have been most likely very "flat," indeed.

In this, as in some other instances, a more "psychologic" rather than "psychometric" analysis of the data was indicated, i.e., evaluation of the items grouped together into certain personality areas rather than in terms of psychiatric scales and syndromes. Before presenting this material, which constitutes the core of the present study, a brief summary of the item analysis carried out with reference to the available scales may be useful.

TABLE 1. MEAN TRANSFORMED SCORES FOR HYPOCHONDRIASIS (Hs) AND INTROVERSION-EXTROVERSION (I-E; A HIGH SCORE INDICATES INTROVERSION). FOR THE ORIGINAL NORMATIVE GROUP THE AVERAGE SCORE = 50, 1 SD = 10. N = 157 YOUNGER MEN AND 223 OLDER MEN, $\triangle$ = MEAN DIFFERENCE, $t = \triangle/SE_{\triangle}$; $t_{0.05} = 1.95$, $t_{0.01} = 2.60$.

Group Scale	Younger Men		Older Men			
	Mean	SD	Mean	SD	$\triangle$	t
Hs	47.6	7.7	49.0	7.1	1.4	1.85
I-E	45.9	7.7	48.1	8.8	2.2	2.59

RESULTS: ITEM ANALYSIS (SCALES)

The total number of items, answered in the diagnostic (scored) direction by a significantly different percentage of the younger and the older men, is indicated for each of the 13 scales in table 2. There is no difference in the number of such items on the F scale. The minimal difference on the L scale is in favor of the younger group; the differences are in the same direction on the K scale, the scales constituting the "psychoneurotic trend" (Hs, D, and Hy) and the Pa scale. There is a negligible difference in the opposite direction on the Pd scale. Clearly separated are the three remaining scales (Pt, Sc, and Ma) in which the younger men tend to give more frequently answers in the diagnostic (scored) direction.

TABLE 2. THE NUMBER OF MMPI ITEMS ANSWERED IN THE DIAGNOSTIC (SCORED) DIRECTION BY A SIGNIFICANTLY LARGER NUMBER OF MEN IN THE YOUNGER (Y) OR OLDER (O) AGE GROUP. T = THE TOTAL NUMBER OF ITEMS CONSTITUTING A GIVEN SCALE. $\Delta = O - Y$. $\Delta\% = \Delta$ AS PER CENT OF T.

Scale*	Age Group		△	T	△%
	Y	O			
L	1	3	+2	15	13
F	1	1	0	64	0
K	2	5	+3	30	10
Hs	2	7	+5	33	15
D	6	15	+9	60	15
Hy	4	8	+4	60	7
Pa	1	6	+5	40	12
M-F	7	15	+8	60	13
I-E	4	9	+5	70	7
Pd	5	4	−1	50	− 2
Pt	7	0	−7	48	−15
Sc	9	2	−7	78	− 9
Ma	13	7	−6	46	−13

*Scales L and F are "validating" scales and K a "correction" scale. They are not named and are identified by a letter alone. Hs = Hypochondriasis, D = Depression, Hy = Hysteria, Pa = Paranoia, M-F = Masculinity-Femininity, I-E = Introversion-Extroversion, Pd = Psychopathic deviation, PT = Psychosthemia, Sc = Schizophrenia, Ma = Hypomania.

It seems that the obtained differences do not demand a consideration of age in setting up the norms for clinical use and that the present standardization on the "general adult population" is valid within wide age limits. We have seen that the difference between the mean score on the hypochondriasis scale did not reach a level of statistical significance (table 1).

RESULTS: ITEM ANALYSIS (PERSONALITY AREAS)

The individual items which differentiated the younger and older men at 5 per cent level of significance or better were grouped in the following areas: Health, interests, sex, work, religion, emotional adjustment, self-confidence, compulsive and obsessive tendencies, norms of conduct, and social attitudes. Each of these categories will be considered in turn. The classification of the item was carried out on the basis of a subjective judgment. No claim is made that the categorization consistently follows a rigorous logical scheme. Obviously, other groupings would be feasible. Some of the present categories could be combined. For practical purposes of verbal description of the results, we have attempted to keep roughly comparable the number of items falling into each category.

The information concerning specific items (percentage of "Yes" responses, the critical ratio of the difference between the two age groups, and the level of significance of the difference) is summarized in table 3. The number of subjects was 79 in the younger group and 223 in the older group. Only the definite answers (Yes or No) were considered, leaving out the very small number of "cannot say" responses. This resulted in a slight fluctuation of the number of subjects on which the percentages were based.

Health.—A general decline in fitness with age and some deterioration of health is a well-established phenomenon. It was present also in our older men who, on ordinary clinical grounds and a detailed study of the cardiovascular system, were considered in good health. They noted more frequently than the younger men such symptoms as buzzing or ringing in their ears, muscular pains, skin sensitivity to touch, and poorer eyesight. About half of them did not "feel" as well as

TABLE 3. ITEM ANALYSIS (PERSONALITY AREAS)

Health

The 5 per cent level of significance of the difference between the frequency of "Yes" responses in the younger and older group is indicated by a critical ratio of 1.97, the 1 per cent level by a critical ratio of 2.60.

Item	% "Yes" Responses		Critical Ratio	Question
	Younger Men	Older Men		
5	16	35	3.06	Am easily awakened by noise.
18	95	82	2.67	Am very seldom troubled by constipation.
29	5	14	2.14	Am bothered by acid stomach several times a week.
62	24	10	3.16	Parts of my body often have feelings of burning, tingling, crawling, or "going to sleep."
63	95	80	3.11	Have had no difficulty in starting or holding my bowel movement.
68	94	83	2.35	Hardly ever feel pain in back of my neck.
155	68	88	3.91	Am neither gaining nor losing weight.
160	80	53	3.98	Have never felt better in my life than I do now.
186	25	10	3.41	Frequently notice my hand shakes when I try to do something.
274	75	32	4.83	My eyesight is as good as it has been for years.
281	94	83	2.29	Don't often notice buzzing or ringing in my ears.
424	20	7	3.06	Feel hungry almost all the time.
425	43	23	3.35	Dream frequently.
533	94	83	2.26	Am not bothered by belching gas a great deal.
541	4	12	2.13	My skin seems to be unusually sensitive to touch.
542	95	81	2.83	Have never had black, tarry bowel movements.

Interests

The 5 per cent level of significance of the difference between the frequency of "Yes" responses in the younger and older group is indicated by a critical ratio of 1.97, the 1 per cent level by a critical ratio of 2.60.

Item	% "Yes" Responses		Critical Ratio	Question
	Younger Men	Older Men		
1	79	66	2.08	Like mechanics magazines.
6	61	36	3.78	Like to read newspaper articles on crime.
70	56	42	2.02	I used to like drop-the-handkerchief.
81	59	47	2.04	Think I would like work of forest ranger.
132	17	39	3.44	Like collecting flowers or growing house plants.
144	16	8	1.99	Would like to be a soldier.
223	76	56	3.09	Very much like hunting.
261	15	34	2.96	Would like to draw flowers if an artist.
283	81	68	2.10	Would like to report sports news if I were a reporter.
428	55	86	5.71	Like to read newspaper editorials.
434	28	7	5.02	Would like to be an auto racer.
537	49	25	3.76	Would like to hunt lions in Africa.
546	62	79	3.06	Like to read about history.
552	89	79	2.02	Like to read about science.
554	36	59	3.27	If I were artist would like to draw children.
561	61	33	4.02	Like horseback riding very much.

TABLE 3. ITEM ANALYSIS (PERSONALITY AREAS) (Continued)

Sex

The 5 per cent level of significance of the difference between the frequency of "Yes" responses in the younger and older group is indicated by a critical ratio of 1.97, the 1 per cent level by a critical ratio of 2.60.

Item	% "Yes" Responses		Critical Ratio	Question
	Younger Men	Older Men		
77	24	37	2.05	Enjoy reading love stories.
133	92	78	2.68	Have never indulged in any unusual sex practices.
231	48	19	3.02	Like to talk about sex.
427	10	22	2.43	Am embarrassed by dirty stories.
430	92	81	2.13	Am attracted by members of the opposite sex.
470	6	18	2.53	Sexual things disgust me.
548	28	42	2.06	Never attend a sexy show if I can avoid it.
558	74	46	4.07	Large number of people guilty of bad sexual conduct.

Work and Work Habits

The 5 per cent level of significance of the difference between the frequency of "Yes" responses in the younger and older group is indicated by a critical ratio of 1.97, the 1 per cent level by a critical ratio of 2.60.

Item	% "Yes" Responses		Critical Ratio	Question
	Younger Men	Older Men		
9	100	88	3.18	Am about as able to work as I ever was.
13	14	56	6.36	Work under a great deal of tension.
90	99	91	2.33	Once in a while I put off until tomorrow what I ought to do today.
148	51	35	2.44	Being interrupted or asked for advice when I'm working on something important makes me impatient.
290	16	58	6.27	Work under much tension.
465	64	46	2.68	Have had change of heart about life's work several times.

they did at earlier periods of their life. At the same time, the other part of the total picture should not be lost from sight, i.e., almost a half of the older men stated that they had never felt better in their life. They did not dream as often as the younger men and they were more easily awakened by noise. Their weight was more stable than that of the younger men.

With age there is a rise in the frequency of gastrointestinal complaints. Constipation, acid stomach, belching, tarry bowel movements, and lesser appetite are among the items which differentiate reliably between the two age groups. Strangely enough, mild paresthesias and shaking hands were noted more frequently by the younger men. It is uncertain at present as to whether these are spuriously "significant" differences, which must be expected to arise by "chance" in certain number of items, or whether they are to be explained on another basis.

Interests.—In their interests the two groups are differentiated along the expected lines.

TABLE 3. ITEM ANALYSIS (PERSONALITY AREAS) (Continued)

Religious Attitudes

The 5 per cent level of significance of the difference between the frequency of "Yes" responses in the younger and older group is indicated by a critical ratio of 1.97, the 1 per cent level by a critical ratio of 2.60.

Item	% "Yes" Responses		Critical Ratio	Question
	Younger Men	Older Men		
58	58	36	2.42	Everything is turning out just like Biblical prophets said.
95	59	47	2.96	I go to church about every week.
249	72	36	5.08	I believe there is a devil and a hell in afterlife.
369	68	85	3.29	Religion gives me no worry.
373	58	42	2.38	Feel sure there is only one true religion.
387	21	36	2.25	The only miracles I know of are simply tricks that people play on one another.
491	26	42	2.30	Have no patience with people who believe there is only one true religion.

The younger men are attracted by strenuous and adventurous activities—hunting, horseback riding, auto racing, the life of a soldier and the forest ranger—while the older men like the bucolic undertaking of growing flowers (and fishing). If they were artists, the older subjects would like to draw flowers and children. They like to read about history while the younger men prefer science and mechanics magazines. In the newspapers the older men like the editorials while the younger men are partial to sports news and articles on crime. Both groups enjoy diversified recreation, although the younger group contains a larger number of men with varied recreational activities.

Sex.—Both age groups acknowledge being attracted by members of the opposite sex, with a slightly lower percentage in the group of older men, who enjoy *reading* love stories but like less to *talk* about sex than the younger men. They are less attracted by a sexy show. Some are disgusted by matters sexual and embarrassed by dirty stories. They believe that many people are guilty of unconventional sexual conduct and one-fourth of the group admits having indulged in what they considered "unusual" sex practices.

Work and Work Habits.—In terms of percentage frequencies, the greatest difference between the two age groups is constituted by the fact that over one-half of the older men work under a great deal of tension. The majority believe that their ability to work has not decreased but they are more compulsive and are less willing, on occasion, to put off matters which should be taken care of immediately. When interrupted during work, they are less impatient than the younger men. Their attitudes toward life's work are more stable.

Religious Attitudes.—The older group is less worried by religious questions. It is somewhat surprising to find that they are less fundamentalistic, less dogmatic in outlook than the younger men—they are less sure that there is only one true religion and that everything is turning out according to the Biblical prophets. A greater number of the older men does not believe in the existence of a Devil and of Hell in after-life. "Miracles" are more likely to be considered mere tricks and church attendance is less regular.

Emotional Adjustment.—On a few items of behavior the older men seem to be more emotional: They consider themselves to be high-strung in a larger number of cases and lose more sleep over worry; they comment more frequently that one or more members of their family are very nervous; they are moved to tears more easily; they are sensitive to criticism and waiting irritates them. But there are

TABLE 3. ITEM ANALYSIS (PERSONALITY AREAS) (Continued)

Emotional Adjustments

The 5 per cent level of significance of the difference between the frequency of "Yes" responses in the younger and older group is indicated by a critical ratio of 1.97, the 1 per cent level by a critical ratio of 2.60.

Item	% "Yes" Responses		Critical Ratio	Question
	Younger Men	Older Men		
80	42	15	4.92	Sometimes tease animals.
128	86	72	2.22	Sight of blood neither frightens me nor makes me sick.
131	71	85	2.64	Don't worry about catching diseases.
138	13	40	4.28	Criticism or scolding hurts me terribly.
158	1	10	2.42	I cry easily.
166	24	47	3.57	Am afraid when I look down from a high place.
181	62	44	2.57	When I get bored I like to stir up some excitement.
238	33	20	2.42	Have periods of such great restlessness that I cannot sit long in a chair.
240	27	45	2.84	Never worry about my looks.
248	51	21	4.74	Sometimes feel on top of the world without reason, even when things are going wrong.
262	80	90	2.24	Doesn't bother me that I'm not better looking.
266	24	10	2.99	Once a week or oftener I become very excited.
268	68	52	2.21	Something exciting usually pulls me out of it when I feel low.
278	46	19	4.65	Have often felt strangers were looking at me critically.
334	19	6	3.50	Peculiar odors come to me at times.
345	16	5	2.86	Often feel as if things were not real.
356	28	12	3.23	Have more trouble concentrating than others seem to have.
367	79	55	3.61	Am not afraid of fire.
392	5	16	2.42	Windstorms terrify me.
396	24	10	3.16	Often feel I don't care about anything even though everything is going fine.
401	81	67	2.25	I have no fear of water.
410	68	46	3.21	Would certainly enjoy beating a crook at his own game.
416	45	20	4.28	Bothers me to have someone watch me at work even though I know I can do it well.
417	25	41	2.58	Am often so annoyed when someone tries to get ahead of me in a line that I speak to him about it.
421	22	35	2.19	One or more members of my family is very nervous.
439	30	59	4.36	Waiting makes me nervous.
442	20	65	6.78	Have had periods in which I lost sleep over worry.
492	16	30	2.41	Dread thought of earthquake.
505	39	17	3.86	Have had periods when so full of pep sleep seemed unnecessary for days.
506	15	31	2.75	Am high-strung person.
510	17	36	3.12	Dirt frightens or disgusts me.
560	21	10	2.32	Greatly bothered by forgetting where I put things.

many more items which indicate the greater emotional responsiveness and presence of emotional tensions in the younger men. They have more frequent periods of excitement and restlessness, as well as of blueness. They are more bothered about their looks and have more trouble concentrating. Their heightened emotionality is reflected in such behavior patterns as stirring up excitement when bored, teasing animals, and enjoying the idea of beating the crook at his own game.

The younger men experience more frequently the feeling that things are not real and that strangers look at them critically.

TABLE 3. ITEM ANALYSIS (PERSONALITY AREAS) (Continued)

Self Confidence

The 5 per cent level of significance of the difference between the frequency of "Yes" responses in the younger and older group is indicated by a critical ratio of 1.97, the 1 per cent level by a critical ratio of 2.60.

Item	% "Yes" Responses		Critical Ratio	Question
	Younger Men	Older Men		
86	16	7	2.10	Am certainly lacking in self-confidence.
100	73	51	3.18	Have met problems so full of possibilities that I have been unable to make up my mind about them.
136	59	33	4.03	Commonly wonder what hidden reason another person may have for doing something nice for me.
142	58	36	3.36	Certainly feel useless at times.
244	38	24	2.29	My way of doing things is apt to be misunderstood by others.
259	31	19	2.08	Have difficulty in starting to do things.
343	20	11	2.07	Usually have to stop and think before acting even in trifling matters.
397	32	19	2.32	Have sometimes felt difficulties were piling up so high I couldn't overcome them.
400	49	27	2.87	Could do things of great benefit to world if given chance.
402	20	58	5.62	Often must sleep over a matter before I decide what to do.
406	42	66	3.62	Have often met supposed experts who were no better than I.
521	77	88	2.42	Would not be embarrassed to start group discussion about something I know well.

Compulsive, Obsessive Attitudes

The 5 per cent level of significance of the difference between the frequency of "Yes" responses in the younger and older group is indicated by a critical ratio of 1.97, the 1 per cent level by a critical ratio of 2.60.

Item	% "Yes" Responses		Critical Ratio	Question
	Younger Men	Older Men		
28	28	15	2.42	When someone does me a wrong I feel I should pay him back for the principle of the thing.
97	15	5	2.02	At times I have a strong urge to do something harmful or shocking.
120	78	64	2.25	My table manners are not quite as good at home as in company.
232	22	46	3.29	Have been inspired to a program of life based on duty which I have since carefully followed.
233	19	32	2.12	Have at times stood in way of people who were trying to do something because of the principle of the thing.
270	63	49	2.17	Don't worry about whether doors locked and windows closed when I leave home.
358	17	4	3.78	Bad words come to my mind and can't get rid of them.
447	67	47	2.98	Often inclined to go out of way to win point with some one opposing me.
458	14	41	4.30	Man who had most to do with me when I was a child was very strict.
467	27	14	2.72	Often memorize unimportant numbers (auto licenses, etc.).
472	18	36	2.89	Am fascinated by fire.
565	2	15	3.00	Feel like jumping off when on high place.

TABLE 3. ITEM ANALYSIS (PERSONALITY AREAS) (Continued)

Norms of Conduct

The 5 per cent level of significance of the difference between the frequency of "Yes" responses in the younger and older group is indicated by a critical ratio of 1.97, the 1 per cent level by a critical ratio of 2.60.

Item	% "Yes" Responses		Critical Ratio	Question
	Younger Men	Older Men		
19	29	12	3.21	When I take a new job, I like to be tipped off on who should be gotten next to.
26	43	63	2.87	Feel it best to keep my mouth shut when I'm in trouble.
89	52	36	2.31	Takes a lot of argument to convince most people of the truth.
117	40	26	2.24	Most people are honest chiefly through fear of getting caught.
135	54	15	6.54	Would probably sneak into a movie if sure I wasn't seen.
141	74	61	2.02	My conduct is largely controlled by the customs of those about me.
250	38	17	3.66	Don't blame anyone for trying to grab everything he can get in this world.
255	39	85	7.37	Sometimes vote at elections for men about whom I know little.
271	36	15	3.86	I don't blame someone for taking advantage of a person who lays himself open to it.
277	42	12	5.55	Have been so entertained by cleverness of a crook that I hoped he would get by with it.
298	51	22	4.53	If several people in trouble, should agree on a story and stick to it.
319	49	31	2.68	Most people inwardly dislike putting themselves out to help others.
378	77	51	3.83	Don't like to see women smoke.
437	43	23	3.22	All right to get around law if you don't actually break it.
444	15	45	4.55	Don't try to correct people who express ignorant belief.
475	54	40	1.98	When cornered I tell portion of truth not likely to hurt me.
481	49	27	3.52	Can remember "playing sick" to get out of something.

They are bothered if they are watched at work. On the other hand, in several respects the older men are less free of fears than the younger group. More frequently they are frightened or made disgusted and sick by the sight of blood. They dread more the elemental forces—water and fire, the violent natural phenomena—windstorms and earthquakes, and are afraid when looking down from a high place. However, they worry less about catching diseases.

Self-confidence.—The material does not yield a very clear-cut and consistent picture. The younger men, when asked point-blank, tend to admit to a lack of self-confidence and the feeling of being useless at times. They are less self-assured in group discussions and fear more that their actions might be misunderstood. They have more difficulty in making decisions when the matters are relatively unimportant. They experience their problems more intensively and believe sometimes that the difficulties they face are too great to overcome. On the other hand, their outlook into the future is hopeful and they feel able to do things of benefit to the world if given a chance.

Compulsive and Obsessive Tendencies.—The compulsion to memorize unimportant data is stronger in the younger men. They cannot get rid of "bad" words and trivial thoughts running through their mind. At times they have the urge to do something harmful or shocking. They will go out of their way to win a point against their opponent. The older men show a greater fascination for fire and cannot tolerate high places. They are more concerned about the safety of their homes and are more rigid in their table manners. The feeling of duty is more prominent. It is of interest to note but difficult to interpret that in a large number

TABLE 3. ITEM ANALYSIS (PERSONALITY AREAS) (Continued)

Social Attitudes

The 5 per cent level of significance of the difference between the frequency of "Yes" responses in the younger and older group is indicated by a critical ratio of 1.97, the 1 per cent level by a critical ratio of 2.60.

Item	% "Yes" Responses		Critical Ratio	Question
	Younger Men	Older Men		
21	25	11	3.07	At times I have wanted to leave home.
52	16	6	2.42	Prefer to pass by school friends or people I haven't seen for a long time unless they speak to me first.
56	4	18	3.11	As a youngster I was suspended from school one or more times for cutting up.
99	60	45	2.20	Like to go to parties and other affairs where there is lots of loud fun.
109	53	34	2.81	Some people are so bossy I feel like doing opposite of what they request, even though I know they are right.
118	20	45	3.85	In school I was sometimes sent to the principal for cutting up.
146	12	5	2.11	I have the wanderlust.
157	13	5	2.15	Feel that I have often been punished without cause.
235	61	79	3.01	Have been quite independent and free from family rule.
237	52	69	2.51	My relatives are nearly all in sympathy with me.
307	19	35	2.61	Refuse to play some games because I am not good at them.
308	28	14	2.88	Have wanted to leave home at times.
386	27	15	2.28	Like to keep people guessing what I'm going to do next.
438	54	31	3.61	Dislike certain people so much I am inwardly pleased when they catch it for something they have done.
454	25	15	1.98	Could be happy living alone in cabin in woods or mountains.
473	15	30	2.55	Avoid being in crowd whenever possible.
495	84	93	2.21	Usually "lay cards on table" with people I am trying to correct or improve.

of cases the person who had most to do with them during childhood is considered very strict.

Norms of Conduct.—The factors determining the younger men's conduct have not become as yet as much "internalized" as in the older group. Their behavior is controlled more rigidly by the customs prevailing in their social environment. They believe that it is the fear of being caught which makes people honest. If it could be done safely, they would be willing to sneak into a movie without paying for the ticket. Truth is a relative matter. When in trouble it is all right to agree on a story and stick to it. A clever crook's actions arouse amusement rather than indignation. Grabbing everything one can get hold of is a widely accepted creed and playing sick to get out of something a not infrequent practice. Most people dislike putting themselves out to help others. In some respects the younger men are the more conservative ones, e.g., the majority do not like to see women smoking but it is the older men that are less inclined to correct people who express ignorant beliefs.

Social Attitudes.—The social adjustment of the younger men is less adequate. They feel more friction within the home and are tempted to get away. They have more bitterness against other people and are likely to do just the opposite thing when ordered around. The older men get along better with relatives and feel more free of family interference. They do not recall having been often punished without cause. Strangely enough, they remember having gotten into trouble during school days for misbehavior more frequently

than the college students. The older men are more likely to lay the cards on the table while the younger men like to keep people guessing about their intentions.

Crowds and loud parties are less attractive to the older men. Putting on a stunt makes them uncomfortable and they are prone to refuse playing a game in which they are not at home. They are less likely to pass an acquaintance without speaking to him and they depend for happiness more on other people; living alone in a cabin is not a very attractive prospect.

DISCUSSION

While the accumulated knowledge of changes in sensory, motor, and intellective functions with age is fairly sound and extensive (2) the trend of alterations in the personality characteristics of the aging man has been chartered much less thoroughly. This is due, in a large part, to the complexity of "personality" and to the severe limitations inherent in present methods. Currently, following the general historical trend in psychology, most of research activity on the psychologic aspects of aging is concentrated in the area of personal adjustment and attitudes (22).

Newer literature on personality characteristics and adjustment has been reviewed by Granick (11). A comprehensive survey of the older literature was made by Kuhlen (18). In the area of age changes in interests of normal individuals, recruited largely from the professions and thus resembling our sample of middle-aged men, the most extensive research was carried out by Strong (23). The results are similar. Between the age of 25 and 55 there is a decrease of interests in the adventurous and out-of-door pursuits, with 55 per cent of the individuals having a positive attitude toward the occupation of the aviator at the age 25, and 16 per cent at the age of 55; for auto racer the percentages were 36 and 9, for secret service man 50 and 27, for "physical skill and daring" 48 and 31. Hunting was "liked" by 76 per cent of the younger men and 51 per cent of the older men. For fishing the percentages were 71 and 57, for playing baseball vs. watching baseball 52 and 19. These percentages reflect attitudes and do not involve actual participation in the pursuits named. There was an increase of interest in such occupations as florist (14 and 31 per cent), real estate salesman (10 and 21 per cent), and social worker (22 and 32 per cent). Also, dislike of change had increased (20 and 35 per cent).

What are the merits and limitations of the present study? They may be considered with reference to sampling, technique of personality description, and evaluation of the data.

The sample is restricted in age to two groups, young adults and middle-aged men. In most investigations reported in the literature the emphasis was placed on the ripe old age; the subjects in Klopfer's sample averaged 74 years (17) and 72 years in the sample studied by Chesrow, Wosika, and Reinitz (8). Such an emphasis has been fully justified in the early stages of research on aging and personality when the gross differentiation between "young" and "old" and the validation of the research techniques with reference to the independent variable (age) has been the primary concern. However, a truly biologic approach demands consideration of personality development through the life span as a whole. Data on maturity, the life period with which the present paper is concerned, are especially scanty.

The present study is cross-sectional in character. We plan to add to it the "second dimension" by repeating the tests and measurements at intervals over a period of 10 years and, if feasible, longer. The focus of the over-all program, of which the psychologic aspects of aging are just one facet, is on the longitudinal follow-up of the older group.

Because of the difficulty encountered in gaining access to "normal' adults, gerontologic research has depended heavily on subjects who are institutionalized. Studies on older individuals of good socio-economic status are infrequent (21). The present study deals with healthy college students (with a good sprinkling of veterans of World War II) and middle-aged business and professional men. Qualitatively the latter group certainly shares the aging tendencies common to the general population. Quantitatively, different segments of the population may age in different functions at somewhat different rates. Available information is not adequate for testing in a rigorous manner such a hypothesis. Considering the selection of the group (absence of disease) and its favorable socio-economic status, the deteriorative changes are very likely to be smaller than would be obtained in a random sample of the general population.

The fact that "personality" is so closely bound with the cultural (20) as well as the social (or interpersonal) and even the natural (or geographic) environment reduces the degree to which results obtained on the given samples may be generalized. Age changes in personal and social adjustment with reference to various occupational groups such as teachers, physicians, office workers, and skilled and unskilled trades are under study at the University of Chicago (4).

In view of the limitations of the staff of any single research institution, the composite psychologic picture of the whole life cycle in the different strands of the population must be pieced together on the basis of a number of studies. This makes plainly evident the need for an adequate characterization of the samples of the subjects used and, whenever possible, the application of standardized tests and measurements.

The Minnesota Multiphasic Personality Inventory (MMPI) was developed as a diagnostic tool of clinical psychology and has been used since its publication in 1942 in a large number of studies. Buros' bibliography (5) cites 72 titles. On theoretic grounds, severe methodologic objections can be raised against all questionnaires as an instrument of research. Ellis (10) wrote perhaps the most devastating criticism of personality schedules, containing 26 theses against the validity of the questionnaires. Yet, in view of our favorable experience with the laboratory use of the MMPI (16) we tend to sympathize with Jenkins' colorful epithet for the questionnaire, "the methodological scapegoat" (15).

The subjects were volunteers who enrolled for a study extending over several years and possibly decades. They were assured of anonymity and of professional handling of the test materials. They were intelligent and cooperative. The whole setting was such as to motivate them toward truthful answers. There was nothing they could possibly gain by a willful falsification. Although all this does not assure the validity of the information provided by the questionnaire, it materially increases one's confidence in it. The internal checks, provided by the "validity" scales L and F, indicate that the responses were not distorted by an undue concern to place oneself in a very favorable light, by a lack of understanding or carelessness.

The inventory consists of a large pool (566) items falling into 26 categories such as general health, complaints referable to the gastrointestinal system, sex attitudes, and morale. The number of items in the several categories varies from 5 (sensibility; genito-urinary system; cardio-respiratory system) to 72 (social attitudes). The scoring scales were not provided for these separate categories but for a number of syndromes recognized in psychiatric nosology. For our purpose the available scales were of very limited usefulness as we were concerned with the description of the age *differences* in attitudes and in behavior rather than with scores along dimensions which would have shown average values very close to those of the normative (normal) adult sample.

In order to describe the multi-faceted human personality it is reasonable to begin with small bits of behavior, such as are represented by the individual items in the MMPI. However, even when we limit ourselves to items which reliably differentiate (in the statistical sense) between the two age groups, the number is too large. Furthermore, such a statistical significance is the beginning rather than the end of the analysis. The differences make biologic sense only if the changes in individual items add up to a more comprehensive, consistent picture. Methodologically, factorial analysis could be used to unravel the relationship between the items and to isolate the main dimensions along which a parsimonious description of personality could be carried out. A comprehensive factorial analysis of questionnaire material has been carried out by Cattell and his associates (6), resulting in an inventory scored with reference to 16 (primary) personality factors (7). The inventory has been administered to our subjects and the results will be reported in a separate study.

The MMPI items were grouped for purposes of economy and order into 10 categories, arrived at on the basis of the subjective separation of the items showing statistically significant differences between the two groups. In the tables the actual percentages of positive answers were given, together with the critical ratios of the differences. Psychologically, both the original percentage values and the differences are of interest. In a verbal description the information about the absolute frequency of the occurrence of a given item is obliterated while a more unified and life-like description is ob-

tained than can be achieved by a simple juxtaposition of single items. For this reason, both modes of presentation have been used.

SUMMARY

1) As a part of an investigation combining the cross-sectional and longitudinal study of aging, with focus on the cardiovascular system, the Minnesota Multiphasic Personality Inventory (MMPI) was administered to a group of college students (mean age 20 years) and a group of business and professional men (mean age 49 years).

2) The average scores on the 3 validating scales were very close to the ideal "normal" values of 50. The mean values of the L ("lie") score were 51.3 and 50.5 in the younger and older group, respectively. On the F ("confusion") scale the means in both groups were 51.6.

3) The inventory was scored with reference to the Hypochondriasis scale (means 47.6 and 49.0) and Social Introversion-Extroversion (means 45.9 and 48.1, respectively); only the latter difference was statistically significant, indicating a somewhat greater tendency toward introversion in the older group. With reference to the general adult population (mean 50, S.D. = 10) both groups, and especially the younger men, were somewhat extroverted.

4) The item analysis was carried out with reference to both the available clinical scales and to personality areas.

5) On most scales the older group answered a somewhat larger number of items in the diagnostic (scored) direction. On Psychasthenia, Schizophrenia, and Hypomania this trend was reversed.

6) Items differentiating the two age groups were considered under the headings of health, interests, sex, work, religion, emotional adjustment, self-confidence, compulsive and obsessive tendencies, norms of conduct, and social attitudes.

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THE RESPONSE OF OLDER RURAL CRAFTSMEN TO INDIVIDUAL TRAINING

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THE SUBJECT of training is one of those matters the importance of which for the satisfactory employment of older workers can hardly be over-estimated. Where replacement of such persons becomes necessary, through health, reduced capacity on the previous job, shifts in production, or others among a variety of possible reasons, successful training will often be essential, if simple movement into low-level, unskilled jobs is to be avoided. Hence it is desirable that as much as possible should be known about the potentialities of the older person as a trainee, what is and what is not practicable, and the conditions which facilitate or hinder his learning of a new task.

Over a period of years a large amount of laboratory work has been carried out on the problems of learning in relation to age and generally the opinion is that a decline of speed or quality, and sometimes of both together, sets in sooner or later during adulthood (2, 3). So far as can be ascertained, this experimental work, using laboratory tasks, has been paralleled by very little study of training of older persons under industrial conditions. Yet information at this point is very necessary, if only to show how far the laboratory results hold under actual working conditions.

A major obstacle to any study of training as it affects the older worker is his rarity among the groups undergoing training in industry or commerce, where it is at least unusual to find any number of people over 40 years old in a training course. Where suitable numbers of older trainees exist, there are not always meaningful records of progress which can be used for analysis, so that it is difficult to obtain much information of the kind required. Particularly is this true for quantitative material; as for assessments of quality, where the course has only one or two instructors their statements about the value of older people as trainees may give a false picture because of unknown biases. Where there are more instructors, dealing with different persons, but teaching the same techniques in the same way, the objection perhaps has less weight. Recently there was an opportunity to gather information under such conditions, and while it is recognized that the material to be presented is subject to many criticisms, in view of the present state of knowledge it is perhaps worth considering.

In England a government-sponsored, but independent, organisation has, as one of its functions, the task of improving the methods of rural craftsmen, such as blacksmiths, engineers, and carpenters, so that they may be better able to meet present-day demands upon their services. A staff of instructors is maintained who spend their time travelling around their allotted districts, giving instruction to individuals or small groups in their normal place of work. The craftsmen can apply for tuition but often a preliminary visit is necessary to persuade them of the advantages to be gained by the instruction which essentially consists of teaching a man who already has a good deal of experience how to execute new techniques in addition to, or in place of, those with which he is already familiar.

A questionnaire planned to obtain information on the effect of the trainee's age on the success of the instruction was circulated by the employing organisation to all of its 26 travelling instructors, and 21 replies were received, covering some hundreds of trainees annually. The questionnaire was drawn up by the present author and a copy is reproduced below.

QUESTIONNAIRE

1. Initials of instructor.
2. Subject in which you instruct.
3. Occupation(s) of persons instructed.
4. What are the ages of the persons you visit; about what proportion of the total is there in each age group? (It is suggested that you might like to use groups such as 31-40, 41-50, etc.)
5. Do you find any *difference between persons of various ages* with regard to the points mentioned under headings A-F below:
   - A. Satisfaction with present methods and willingness to be taught new ways.
   - B. Understanding of the material you teach them.

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C. Speed of learning, or the number of visits required before the material is fully learned.
D. Questions they ask during the teaching.
E. Putting what has been learned into practice.
F. Minor accidents (such as cuts, etc., needing any kind of attention) while practising the new methods.

6. If you have any other comments which you think would be helpful, please add them here.

RESULTS

The greatest number of replies (13) came from teachers of welding and other engineering techniques, with 4 from woodworking machinery and 4 from costing and bookkeeping instructors. While the replies from each category of instructors did not show any marked difference, the costing and bookkeeping tutors are trying to impart skills which are different from those of the other groups, and so their replies were kept separate when the various parts of Question 5 were considered. Before going on to discuss the answers it is, however, necessary to consider at this point the present weight of the methodologic difficulties outlined by Ruch (4), those relating to selection, motivation, and the control of initial ability.

First of all, it is quite clear that the population in an industrial training course is not controlled with regard to any of these three points in a way which would be regarded as satisfactory in a laboratory experiment. As a result, there are serious limitations to the amount of knowledge which can come from this source, as regards the real changes in learning ability with age. However, it is most unlikely that the industrial instructor will ever have to teach a population whose component age groups are evenly weighted in respect to factors of motivation, selection, and initial ability. The study of industrial and other courses may, then, be able to contribute something to the improvement of existing methods and the extension of training into age groups above those principally involved at present. In this study, selection may have had a greater influence on the older age groups, through the elimination of poor performers over the years, and insufficient is known about the variation in initial ability at the tasks to be learned. Motivation is one of the subjects of enquiry, but it should be noted that persons who did not want instruction, or could not be encouraged to have it, were under no obligation to learn the new techniques, and it may well be that those who did not take instruction were mainly older men.

Ages of Trainees. (Answer to Question 4).—The instructors were asked to estimate the proportions by age groups of the persons visited, but although decades were suggested as convenient units, the answers were very varied in the way that numbers and ages were given, and one person did not answer this question. It was, however, quite clear that an unusually wide age range of persons was being taught, from juveniles to men of 50 or over, and in some cases up to the age of 65. Eleven replies included figures which could be used to calculate a rough proportion for trainees over 40 years old, who seemed to form about 35 per cent of all those instructed, the separate estimates varying from 20 per cent to 60 per cent.

Observed Differences Between Ages (Answer to Question 5).—Answers to the various parts of Question 5 varied a good deal, and two of the questions, those concerning accidents and the queries of trainees, had particularly low numbers of appropriate answers. The remarks about questions asked by trainees (5 D) made by most of the teachers did not have any age reference, and this was most probably an indication of difficulty in making any distinctions. The question about accidents (5 F) was not applicable to the costing instructors, and 9 of the others said that accidents were too rare for judgment to be passed. As the remaining answers taken together did not show any favour towards or against older trainees in this respect, the matter need not be considered further.

As the numbers of answers were not large enough for statistical treatment, a suitable method seemed to be grading them into (I), those reporting no decrement with age, (II), some decrement, (III), decrement to a degree serious enough to throw doubt upon the value of the training, and (IV), statements which do not bear upon the issue. The distributions of each kind of answer are shown in table 1, those for costing instructors being given separately.

It will be noted that the costing instructors were both more definite in their answers, as shown by the absence of irrelevant statements, and more uniform in the kind of report given, than were the other tutors. In

TABLE 1. CLASSIFICATION OF ANSWERS ACCORDING TO REPORTED DECREMENT WITH AGE.

Question. (Parts of Question 5)	Number of Answers							
	Engineering and Woodwork Instructors				Costing Instructors			
	Ratings*				Ratings*			
	I	II	III	IV	I	II	III	IV
(A) Willingness to learn new ways	6	6	4	1	1	2	1	—
(B) Understanding the teaching	7	2	—	8	—	3	1	—
(C) Speed of learning	3	9	2	3	—	3	1	—
(E) Putting into effect	5	5	2	5	—	4	—	—

*I. No decrement.
II. Some decrement.
III. Serious decrement.
IV. Irrelevant answers.

part this may be attributable to the former group of teachers being more accustomed to dealing with written material, but there may also be a genuine difference in the kinds of response to training. In particular, there is a suggestion that the likelihood of an older person getting through the training as well as a younger one is less for the costing and bookkeeping skills than for the engineering or woodworking techniques. However, since the latter group of skills is much the more likely to be related to the trainee's previous experience, any difference that exists is not simply to be attributed to the difference in the nature of the skills required.

While the suggestions of crucial difficulty with older persons are few in number, there could be a tendency to understatement on this aspect, since such a report might be construed as a reflection upon the capability of the instructor. However, all in all, reports of some decrement were frequent and merit further examination, question by question.

(5A) Satisfaction with present methods and willingness to learn new ways. It is clear that reluctance to change methods of work, even when these were no longer adequate to meet changes in the kinds of jobs to be done, was common among the older craftsmen, although statements to this effect were sometimes qualified by reference to elderly individuals who were keen to learn new techniques. While persons who steadfastly refused to learn anything new may have existed, it seems that the men who were reluctant at first could usually be persuaded to take the instruction.

(5B) Understanding of the material taught. —The use of the word "material" seemed to have misled some of the tutors, since it was sometimes taken as meaning the substance used in the work, from which follows the relatively high proportion of irrelevant answers. Apart from these, the opinion of the "technical" instructors preponderated in the direction of no change with age, and it seemed that positive transfer from previous experience in the general subject was important as an aid to comprehension. On the other hand, all of the costing teachers reported a decline with age in ability to understand the tuition, one stating that "the younger men understand but the older follow the methods blindfold," and another that theoretic principles were grasped more quickly by the younger pupils. The trainees' unfamiliarity with the subject, by comparison with those taught by other instructors, and the larger role of theory as against practical demonstration, seem to be the major factors involved; both have been

seen to operate against older trainees elsewhere in industrial and commercial training schemes (5).

(5C) Speed of learning.—A decline with age in the speed of learning seemed to be widespread, and often necessitated an increased number of visits to the older men, but detailed examination of the answers throws little light upon the complexities of this problem.

(5D) Questions asked.—A reluctance on the part of older people to ask questions was mentioned twice, as was a tendency for them to ask about practical applications rather than about theoretic issues, the latter being raised more frequently by younger men.

(5E) Putting into practice.—To judge from the reports, there was some resistance by older persons to using the newly acquired techniques instead of the old ones in their normal work, but only rarely was this so serious as to throw doubt upon the value of training them. Once again, all the costing instructors were among those reporting difficulty, and in addition one pointed to an age difference in the way of application, older people doing so to the detail, many younger ones with variations to meet individual requirements.

Any other comments (Answer to Question 6).—Here remark was made upon the high degree of variation between individuals and the good performance of some older trainees. The point was made that resistance to new methods was often related to doubts concerning the repayment of money invested in equipment by the resulting increase in earnings or lowering of costs during the limited number of working years which the man had left. Once again reference was made to a greater decrement with age in understanding theoretic teaching than in following practical demonstrations.

DISCUSSION AND CONCLUSIONS

Age at which difficulty appears.—In the foregoing discussion, the terms older and younger have been used without specific age reference, as was usually the case in the replies. However, if we wish to assess the age at which difficulties in training of this kind make their appearance, 8 instructors put the level at 40, 2 at 45, 3 at 50, and 8 did not give approximation of such age.

Age of instructor and nature of answers.—A check was made of the relation between the age of the tutor and the kind of report he sent in. Of the 21 instructors, 2 were aged 21 to 30, 7 were 31 to 40, and 11 were 41 to 50, the oldest of all being 52. There seemed to be little difference in the kind of reply up to the age of 45, but above this the instructors gave a large number of irrelevant answers, a relatively high proportion graded I and none graded III. It is fair to conclude that these men were giving reports more favourable to the older trainees than were the younger instructors, and there are three possible explanations of this tendency. Either, because of their own age, they were inclined to play down difficulties observed among the older trainees; the younger teachers tended to over-emphasise such weaknesses; or the older instructors were in fact able to get a better response from elderly pupils. While it is highly likely that older people will tend to have a relatively good opinion of the capabilities of persons of like age, the possibility that the use of older teachers would have beneficial results is interesting enough to merit further checking.

Assessment of results.—It is important that the restricted scope of the enquiry should be borne in mind when considering the import of the results described; other types of training for very different skills might well show results opposed to the present ones. Indeed, Belbin (1) found that training difficulties with age were frequent on jobs involving high speed manual skills, and appeared early, in some cases by the age of 30. In such tasks training is not only concerned with learning the method but with the completion of each cycle of movements in a very short time. Similar difficulties were reported to Shooter (5) from a factory which had attempted to train older people on several semi-skilled tasks involving short, rapidly-recurring cycles. The type of course which figured in this study was concerned with aspects of a general field with which the trainees were already familiar, for which they already had a range of skills, and with techniques taking a relatively long time—minutes at least—to complete.

While remaining aware of the restricted scope of the investigation, we may ask the question of what is likely to be the outcome of an attempt to train older persons by the

method which these instructors were using. The answer depends on which of two criteria is to be used—the existence of negligible decrement over a wide age range, or the presence of some disability which can be alleviated by appropriate action. In general, this corresponds on the one hand to grade I (no decrement) against grades II (some decrement) and III (crucial difficulty), and on the other hand to grades I and II against grade III. The results given in table 1 can then be used to find the scores for each criterion on the various items of the questionnaire. The general indications are that it will be necessary to accept the existence of some limitations, particularly with regard to initial resistances and lessened speed of learning, but that the difficulties are not insuperable and can be overcome by suitable adjustments of the training scheme. Further study is urgently needed to show the possibilities for training older people, where the limits lie, and how far changes in the methods and circumstances of training can help in extending the range of practicability.

SUMMARY

Persons engaged in the individual instruction of rural craftsmen in modern techniques were given a written questionnaire on the effect of age on various aspects of the training. The 21 replies received are analysed to point out the extent and locus of difficulty among older men, showing that although older people were rarely untrainable under the conditions in operation, there was frequent resistance to learning new techniques and a lower rate of learning. Although the data are severely limited and only cover one specialised type of instruction there are several indications of ways by which the learning of older people can be facilitated.

This study was made possible by the cooperation of the instructors concerned and that of other officers of their organisation. The author also wishes to thank Mr. A. T. Welford, Director of the Nuffield Research Unit, and Dr. J. Szafran, Deputy Director, for their valued suggestions and criticisms.

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SURVIVING THE FIRST FIVE YEARS OF RETIREMENT*

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DOES ENTRY into retirement shorten life expectancy? In recent years the assumption that adjustment to retirement imposes a lethal strain on many, if not most, individuals has become widely accepted. Those who endorse this view offer in logical support that upsetting of work routines, loss of status and prestige, the breaking of established social relationships, and other consequences of retirement place a severe mental stress upon the individuals concerned. Frequently this mental taxation is translated into physical disorders which may prove fatal before adjustment can be made. Once the critical adjustment period has been passed, chances of survival improve or at least become more normal. As statistical evidence, proponents of the "retirement impact" theory point to the excessive mortality at ages 65 and 66 of workers retiring under the old-age survivors insurance program, and similar patterns for other retired groups (1).†

MATERIAL AND METHODS

Because of the general interest in the effects of retirement upon longevity, the "retirement impact" proposition has been tested by utilizing a population of retired Army and Air Force officers. While the total population consisted of officers who spent some part of their life in retirement during the period 1925-1948, two sub-groups of the population were used in attempting empirical verification of the hypothesis. The first group consisted of 2,798 officers who died while in retirement during the 24-year period. The second group consisted of 1,432 officers who retired during the period 1925-1933—the source of the data was (2). Both groups were classified by age at retirement (in 5-year age groups) and number of 5-year periods lived in retirement up to and including three periods, or 15 years. From these tabulations "successive survival ratios" were computed. To illustrate, if 80 of 100 officers retired at ages 60-64 survived the first 5 years of retirement, the survival ratio for that initial period was .80. If 60 of those 80 survivors lived through the next 5 years, the ratio for the second period was 60/80, or .75.

It was reasoned that if entry into retirement is a factor increasing mortality the survival ratio for a given age group should be lowest in the initial retirement period. For example, there should be a smaller proportion of survivors of a group aged 60-64 passing through its first 5-year period of retirement than of a group in the same age bracket passing through its second 5-year period (having retired at ages 55-59). Even if the higher mortality rate were experienced only during the first 1 or 2 years of retirement, it should serve to lower the survival ratio for the full period since there is no reason to expect that the mortality during the remainder of the period is markedly lower than average.

RESULTS

Survival ratios for the deceased officer group are shown in table 1. Four 5-year age groups were compared: 50-54, 55-59, 60-64 and 65-69. For 3 of the 4 age groups, survival ratios were lower in the first period of retirement than in the second, for 2 of the 4 groups, ratios were lower in the first period than in the third. Only 3 of the ratio differences were statistically significant, however, and all these involved low survival ratios obtained for the third retirement period. (Chi-square was chosen as the test of significance. Some question was raised in the minds of the authors, however, as to the adequacy of the chi-square distribution to represent expected

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*Personal views or opinions expressed or implied in this article are not to be construed as necessarily carrying the official sanction of the Air Research and Development Command or of the Department of the Air Force.

†Myers, whose article is referenced as (1), does not accept the "retirement impact" theory, however, noting that the data are not specific or reliable for that purpose. In his summary he states, "it seems likely that this higher mortality in the early years of retirement arises from the fact those in poorer health are more apt to retire at or shortly after the minimum retirement age, while the healthier individuals continue at work."

TABLE 1. SURVIVAL RATIOS BY AGE AND 5-YEAR PERIOD OF RETIREMENT FOR DECEASED RETIRED OFFICERS OF THE UNITED STATES ARMY AND AIR FORCE, 1925-1948.

Age	5-Year Period of Retirement					
	First		Second		Third	
	Base Number*	Ratio	Base Number	Ratio	Base Number	Ratio
50-54	496	72.6	244	77.9	128	73.4
55-59	516	66.3	360	68.9	190	68.4
60-64	839	74.1	342	69.0	248	66.5
65-69	86	67.4	434	69.8	129	54.7

*Base number is number of officers of age group entering specified period.

variations. The development of an empirical curve against which to test such distributions is under contemplation.) Specifically, the proportion of officers 60-64 who survived their first 5 years in retirement was significantly greater (at the .05 level) than the proportion in the same age bracket who survived their third 5-year period. In the 65-69 year age group, significantly higher proportions survived both first and second 5-year periods than the third 5-year period. The first period survival ratio was significant at the .05 level; the second period ratio was significant at the .01 level. It is highly probable that the lower survival ratios during the initial periods for the 50-54 and 55-59 year age groups are attributable to higher proportions of disability retirements. The 60-64 year age group had the smallest proportion of disability retirements and showed the highest survivorship during the initial period of retirement. It is difficult to account for the relatively higher mortality experienced in the third 5-year period of retirement by the two older age groups. It is counter to the experience of other retired groups in which mortality rates became more "normal" as time in retirement increased, indicating progressive dilution of the effects of disabilities which may have caused initial retirement (1).

In order to obviate biases introduced by utilizing data on a deceased population, the second group chosen was a retirement cohort consisting of all officers who retired during the 9-year period 1925-1933. This period was chosen so that all of the officers would have either died or spent at least 15 years in a retired status by the end of 1948. Survival ratios for this group are given in table 2. The survival ratios for this group are, of course, higher than the comparable ratios for the deceased group. Furthermore, the trends by survival period are different. For every age group survival ratios are greater for the initial 5-year retirement period than for the second 5-year period. For the two younger age groups, 50-54 and 55-59, ratios are lower for the initial period than for the third 5-year period. None of these differences proved statistically significant, however. It is interesting to note that the age group 50-54 had a higher rate of survival during the initial retirement period than any of the older age groups in the same period, although this youngest group probably contains the highest proportion of disability retirements.

As an additional check, survival ratios for this retired officer population were compared with survival ratios computed from the life table for U. S. white men, 1939-1941. Based on the 1939-1941 life table for U. S. white men, 92.2 per cent of the age group 50-54, 88.7 per cent of the age group 55-59, 83.9 per cent of the age group 60-64, and 77.1 per cent of the age group 65-69 should have survived the next 5 years. For the initial period, officer survival ratios were significantly lower than expected for age groups 50-54 and 55-59

TABLE 2. SURVIVAL RATIOS BY AGE AND 5-YEAR PERIOD OF RETIREMENT FOR OFFICERS OF THE UNITED STATES ARMY WHO RETIRED 1925-1933.

Age	5-Year Period of Retirement					
	First		Second		Third	
	Base Number*	Ratio	Base Number	Ratio	Base Number	Ratio
50–54	317	88.6	110	86.4	102	90.2
55–59	277	82.3	281	81.1	95	84.2
60–64	326	86.2	228	82.5	228	83.3
65–69	†	....	281	77.9	188	76.1

*Base number is number of officers of age group entering specified period.
†Base less than 50; of 15 officers retiring in this age group 12 survived for a ratio of 80.0.

but significantly higher than expected for age groups 60-64 and 65-69. Although our retired populations are small, this probably indicates the selectivity of the retired groups. The disabled retire at earlier ages and die in greater numbers shortly after retirement. The healthier officers retire for age or length of service and live longer. For the second 5-year period of retirement only the oldest group of officers had a higher survival ratio than that computed for U. S. white men of comparable ages. None of the age groups in the third period of retirement had survival ratios as high as those computed from the life table. Again, this would seem not to agree with the principle of the "wearing off" of the effects of retirement disabilities with the passage of time, although there may be a reasonable explanation.

DISCUSSION

The evidence from the two groups of retired officers does not support the proposition that entry into retirement is an especially deadly transition. It is true—at least for the tested population—that the mortality of officers retired before they have reached 60 years of age is higher during their first five years of retirement than might normally be expected on the basis of life table data for U. S. white men. But this is most probably due to the fact that early retirement frequently results from physical disability, and the latter is responsible for the high mortality. There is no indication that officers who retire at age 60 or later are adversely affected by entering into a retired status so far as their longevity is concerned. In fact, the evidence is to the contrary—that their chances of survival are greater than those of the average white man of comparable age. The explanation is probably to be found in physical selectivity, however, rather than in any salubrious effects of retirement.

Officers of the studied populations who entered retirement at ages 50-54 and 55-59 were generally less likely to survive five years than officers of comparable ages who had retired earlier and were entering their third 5-year period of retirement. However, officers entering retirement at age 60 or later were considerably more likely to survive their first five years of retirement than earlier retired officers of comparable age were to survive their second or third periods. While this disparity is probably due in considerable measure to the better than average physical condition of officers retiring at later ages, it also seems attributable in part to the below average survival of groups retiring before age 60, regardless of length of time spent in retirement.

SUMMARY

This study was designed to measure the mortality effects, if any, of entry into retirement. The tested hypothesis was that sur-

vival rates are lower for given age groups during the first 5-year period of retirement than for comparable age groups in two subsequent 5-year periods of retirement. Two test populations were drawn from a universe consisting of all Army and Air Force officers who spent any time in a retired status during the period 1925-1948. The first group consisted of 2,798 officers who died while in retirement during the period. The second group consisted of 1,432 officers who retired during the period of 1925-1933. Evidence from the two test groups failed to substantiate the proposition that survival rates are consistently lower for the initial period of retirement. Hence, it cannot be concluded that the "impact of retirement" shortened the life expectancy of the population.

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STANDARDIZING AN INDIVIDUAL INTELLIGENCE TEST ON ADULTS: SOME PROBLEMS

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THE PROBLEMS which face the author and publisher who set out to standardize a test on an adult population vary in complexity, intensity, cost, and time consumption according to the nature of the instrument and the rigor of the standards they, the author and publisher, impose on themselves. This paper is concerned with one of the most ambitious attempts of its kind—the standardization of the Wechsler Adult Intelligence Scale. Examples of the problems which have been met are drawn from two projects—from the standardization proper and from a separate study of the aged.

The standardization of WAIS, as the test is usually called, was in many ways unique. The very attempt to obtain a representative sample of the adult population of the United States with an individually administered test was unusual if not unprecedented. What has been achieved represents, it is believed, the best norms that have been collected in this type of situation—although obviously and admittedly they are not perfect. Some of the limitations of the data will become evident in the discussion of the problems which arose.

Before embarking on the standardization testing, two sets of basic decisions had to be made. The first set had to do with defining the population to be sampled. It was necessary to decide:

a. *What age range should be included?* It was agreed after some discussion to test people from 16 to 64 years of age in the following age groups: 16-17, 18-19, 20-24, 25-34, 35-44, 45-54, and 55-64.

b. *What ethnic groups should be included—whites only, or all groups?* The decision was reached not to limit the sample to whites only; whites and non-whites were specified according to their proportions in the national census.

c. *How many should be tested?* Clearly, the larger the number of cases included, the better for the sample. Equally clearly, the administration of an individual intelligence test to selected subjects is an expensive process; furthermore, the setting of careful controls over the sample to be tested is more important than mere addition of numbers of cases. The controls make it more difficult, and more expensive, to obtain cases, but they give the resulting sample far greater meaning. The numbers finally agreed on were: 200 per age group for the 3 youngest groups, 300 per age decade between 25 and 54, and 200 for the group between 55 and 64, the total being 1700 cases.

The controls to govern the sample to be chosen had next to be decided on. The following controls were finally set forth, each control applying within every age group.

a. *Sex.* An equal number of males and females were to be included.

b. *Education.* Educational level was categorized as: 0 to 8 years of schooling, 9 to 11, high school graduation, 13 to 15 years of education, and college graduation or more advanced study.

c. *Occupation.* Census categories were utilized to form 13 occupational classes, from professional and technical people through clerks and craftsmen, farm laborers and foremen, to students, housewives, and others not in the employed labor force.

d. *Urban-rural.* Communities were classed as rural if they had less than 2500 population and if they were not adjuncts of larger cities.

e. *Geographic region.* Census groupings were used to divide the map of the United States into 4 geographic areas: northeast, north central, south, and west.

These decisions having been reached, the project came up against the first operating problem: how to specify the characteristics of every one of the 1700 individuals to be tested.

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The U.S. census data for 1950, although vital as a source of information, do not offer tables which embody the several cross-control variables we have imposed. It was not an easy task to prepare charts specifying for each individual his relevant characteristics in such a way as to maintain, as closely as possible, representativeness of the total sample on each of the controls.

When the charts were finally made, it was possible to give each examiner exact specifications for every subject he or she was to locate. For example, one examiner was instructed to test a non-white, rural female, 20 to 24 years of age, engaged in housekeeping in the north central area, and with eighth grade education, or less. Another examiner was asked to test a white male urban proprietor or manager, 35 to 44 years old, with between 9 and 11 years of schooling.

With so many specifications set down for each prospective subject, much time and effort were needed to locate an individual who met all the requirements. But even after an examiner had succeeded in locating a subject who met every specification the subject might refuse to be tested.

Herein lies one of the major operating difficulties involved in standardizing tests on adults rather than children. In standardizing the Wechsler Intelligence Scale for Children it was necessary only to obtain the cooperation of school authorities, who did not have to act as subjects themselves and who were sympathetic with intelligence testing. Once the school's acceptance was had, compliance by children followed; the cases were selected, scheduled, and tested.

The situation was quite different for the adults. The subjects were not always familiar with, or sympathetic with, intelligence testing. Furthermore, since they were themselves to be the persons taking the test, they often seemed to feel threatened by it. As a result, many prospective subjects refused to take the test. Whenever possible, of course, the examiner tried to persuade recalcitrant subjects to relent and comply; sometimes the examiner was successful, sometimes not. When unsuccessful, the examiner then had to seek another prospect who met all the specifications and would agree to be tested.

This matter of willingness of the subject to be tested is of considerable importance even beyond the additional time, expense, and frustration resulting from refusals. A more important consequence is the bias in the representativeness of the sample. It is logical to believe that, on the whole, people who are unwilling to take an intelligence test are less intelligent than their peers who are willing to be examined. A precise estimate of how much bias there is would be difficult, if not impossible, to obtain. That it does exist, and the direction in which it operates, can nonetheless be assumed.

There is another bias which should also be recognized as influencing the sample. This has to do with a kind of cultural selection which is personal to the examiner. It is only natural for an examiner to reach out for subjects from those around him—relatives, friends, and acquaintances. The examiners used in the standardization were all professional people, with above average education. Their friends and acquantances, it may be expected, were therefore likely to be people who are superior to their own educational and occupational peers. For example, if the writer were asked to test a white craftsman, aged 35 to 44, with 12 years of education, he would draft a carpenter friend who happens to meet these requirements. His friend would be available and compliant; but he would not be representative, in intelligence, of 35 to 44 year old craftsmen with similar education. The writer has found him a congenial companion because he is above average—in knowledge and in intellectual curiosity. It is probable that at least some of the subjects who were most readily available to the examiners were similarly unrepresentative. Again, it is not feasible to compute how much of an effect this congeniality bias has on the sample; it can only be assumed that the bias does exist and operates to increase the raw score required to obtain a given IQ.

The problems noted above (and a number of difficulties not discussed herein) applied to all the age groups in the standardization proper. There were some problems, however, which were particularly troublesome in testing older adults. These may perhaps be best illustrated by reference to a supplementary project, the attempt to extend normative data to older people than those in the regular standardization. Through a grant from the U.S. Public Health Service, the Commission

on Human Development of the University of Chicago has been engaged in intensive study of the aged in Kansas City. The cooperation of Dr. Havighurst permitted the tying in of WAIS testing with the larger investigation. The subjects to be tested were the adults aged 60 and over selected by means of an area probability sample drawn from the metropolitan area of Kansas City.

Essentially, the program for obtaining cases was opposite to that used in the main standardization. In the Kansas City study, the subjects were specified in terms of their addresses, and the examiner had to contact the specific examinee, to persuade him or her to participate, and then to administer the test. The problem of *locating* subjects, so troublesome in the standardization proper, was less troublesome in the study of the aged.

This did not mean that the task of the Kansas City examiners was easier than that of examiners in the national study. Although comparative data are lacking, it is reasonably certain that as compared with younger adults, the older potential subjects were, on the average, less familiar with the whole idea of testing and therefore less sympathetic; they were also more likely to be suspicious of the examiner's purposes. The greater age difference between the elderly subjects and the examiners might well have made rapport more difficult. More of the advanced in age were obviously less able physically to make the simple movements involved in the performance portion of the scale; many of these potential subjects nevertheless did the verbal tests and one or more of the performance tests; this is known since their records are available. Quite probably, many other of the physically less able refused to take any part of the scale, once they recognized the kinds of tasks they would be asked to try, although they may have given other reasons for their refusal. Whatever the reason given, it often meant that the subject simply was not tested, despite the continued efforts of one or more of the examiners.

One of the interesting sets of data which were compiled in this study was a record of why prospective subjects were not tested. The examiners, while obtaining complete tests on 352 subjects and less complete tests on 123 more, were unable to test 182. Of those not secured, 90 were men and 92 women. Reasons for failure were classified into 5 categories. One category—refusal—contained half (90) of the failures; 57 other subjects not secured were either too ill or deceased. The remaining 35 failures were accounted for by such factors as having moved from the city, being unable to understand English, etc.

Some of the subjects' reasons for refusing may be illuminating. The remarks come from report forms filled out for each individual from whom a test was not obtained: A 61 year old housewife contacted by 2 examiners on 5 different occasions said she did not believe in surveys; too many people came to the door; she was just not interested. A 70 year old retired railroad employee, contacted by 3 examiners on 4 occasions said: "If you were selling $10.00 bills for $1.00, I wouldn't be interested." He also remarked, rejecting the examiner's plea, that he was not interested in helping other people. A 64 year old decorator (2 examiners, 3 contacts) said, "I know what you're trying to do. I've been in the selling game too."

The 75 year old wife of the president of a motor company said she did not want to be in the public eye and would hear no explanation; a 61 year old wholesale clothing salesman, learning it was an intelligence test, said he would have no part of it, that he had taken intelligence tests before and knew all the answers; a 67 year old factory operative thought the test was too personal; an 86 year old retired music teacher would say only that she hated everybody except her mother.

There was a woman, described in a special report prepared by one of the examiners as the most paranoid subject encountered, who "ordered the examiner out of the house because of some 'plot' that her sister and the government had contrived against her. Paranoid tendencies were further indicated by the numerous crucifixes hanging from the mailbox, the many crucifixes and religious pictures hanging on the walls, a large faded wreath hanging among the crosses, the furniture swathed in white cloth, the shades pulled down, a heavy mantle of dust coating the interior of the house, and the woman herself wearing numerous strings of rosaries around her neck."

No mention has been made of problems with respect to the conditions under which testing was attempted. Omitted also are problems of test theory and test construction—

should time limits be removed, should separate norming techniques be devised, and similar matters of importance and concern. Problems like these nonetheless are real and present, and must be anticipated by anyone who would work in this field.

Perhaps the best advice which can be offered to those who would try similar studies is: be financially prepared for heavy drains on the exchequer; be intellectually prepared to exercise all the ingenuity—your own and your colleagues'—at your command; be emotionally prepared for frustration and the necessity for compromise. Above all, be grateful to the hundreds of men and women who do cooperate with you, to examiners who adapt themselves to working at unusual hours under often difficult conditions as well as to the subjects who, often not even understanding the purpose of your study, give freely of their time and effort to forward your work and contribute to society's knowledge of itself.

SUMMARY

A project aimed at the standardization of an individual intelligence test on a representative sample of adults in the United States—and especially if the research is to include the aged as well as younger adults—must be prepared to cope with problems of:

1. Financial cost — beyond expenditures which are ordinarily possible by an individual.
2. Research facilities—for the production of materials and analysis of the data.
3. Cooperation of competent colleagues to supervise and examine the subjects.
4. Description of a sample of adequate representativeness.
5. Selection of the specific individuals to be tested.
6. Persuasion of the selected individuals to agree to be tested.

If the first three problems cannot clearly be resolved, the project should hardly be undertaken at all; the success with which the last three are met will determine the validity of the norms—the goodness of the job.

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BOOK REVIEWS

EDUCATION FOR LATER MATURITY, Wilma T. Donahue, editor, Whiteside, Inc., and William Morrow, Inc., New York, 1955, 338 pages, $4.50.

This is the first book ever published devoted exclusively to *education* for later maturity. Planned by the Committee on Education for Aging of the Adult Education Association of the U.S.A., it is the work of a group of persons under the leadership of the chairman of the Committee, Wilma Donahue.

The first two chapters provide an excellent framework for what follows. There is some very good philosophy in Chapter I by Lawrence K. Frank. He makes an excellent case for education for later maturity for democracy's sake. The wisdom and accumulated experience of the older population, he says, are largely outdated and no longer related to the new problems and conditions of contemporary life. That, and the fact that so many of them are burdened by handicaps and impairments, are insecure and unhappy, make them responsive to appeals of popular leaders to organize pressure groups. This potential threat happens at a time when significant changes are taking place in our economic, political, legal, social, and family patterns and when too many of our older people have lost their capacity for new learning and readjustment and are resistant to change. A limited capacity for new learning is not the inevitable effect of aging and can be avoided. This chapter, it might be said, presents the purposes and promises for education in the later years.

Clark Tibbitts, in Chapter II, discusses "Aging in the Contemporary Scene." He gives some significant statistics which point up the need for new roles and opportunities. He tells how adult education can play a vital part.

Two of the very best chapters, in the reviewer's opinion, are those on "Capacities of Older Adults" by Irving Lorge, and "Teaching and Learning" by John Anderson. Lorge emphasizes the difference between *learning rate* as efficiency or performance; and *learning ability* as power or potential—a distinction which is fundamental. "The observed declines in sensory acuity, in physical speed, and psychologically adapted tempo, lead to underestimates of learning ability, and by analogy, from such declines, to underestimates of intelligence as well." . . . "Age, as age, probably does little to affect one's power to learn." Intelligence is power, not speed, and intelligence does not decline.

Anderson, in Chapter IV, gives an excellent summary of principles of learning and teaching, showing that they are essentially the same for young and old. Underlying any theory of the education of the older person is the fact that he and his relations with others change as he grows older. These changes come, first, from the aging process itself which affects vision, hearing, etc.; second, from society; and third, from group expectancies as to what the older person should or should not do, and these create adjustment problems. The primary concern of an educational program for older adults is with interest, skills, and attitudes that make possible a richer and happier life. Four purposes are given for an educational program: 1) to give an understanding of the changes taking place and an awareness that one is facing these problems in common with others; 2) to impart knowledge and skills to maintain health, retain or increase mental capacity; 3) to give satisfaction in learning for itself; and 4) to provide a richer social experience and better understanding of the world.

The steps in learning, Anderson says, are similar in all ages, but attitudes toward learning change. Older people are more concerned with quality of learning and the effect of what they do in terms of others, and they are sensitive to what others think. He gives a very fine summary of similarities between young and old in learning and the differences which the instructor should consider in his teaching.

The longest chapter, that on "Educational Programming," is somewhat spotty. Some portions of it, such as adult education in the public schools and libraries, are excellent. Some sections could well have been omitted for they contain nothing specifically on education for later maturity. For example, the discussion on "Opportunity Schools" is not on opportunity schools in general but on a particular school, the Denver Opportunity School, and the writer does not mention any programs specifically for older persons.

The section of the chapter on Federal agencies likewise contains little on educational programs specifically for later maturity.

In spite of the shortcomings in the chapter on educational programming, it is a very helpful one and will provide considerable practical help to those planning programs in education for the older population.

The Adult Education Association is to be complimented on its very real contribution to an increasingly important aspect of adult education.

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OLDER WOMEN AS OFFICE WORKERS, Bulletin of the Women's Bureau, No. 248. Published by the U. S. Government Printing Office, 1953, 25 cents.

This Bulletin is in two parts. Part I, "Training Program in Four Cities," reports the experience of four communities (Denver, Colorado; Cleveland, Ohio; New York City, and Milwaukee, Wisconsin) in carrying on pilot projects designed to help meet the labor market problem resulting from reluctance of employers to hire women over 35 for office work in the face of shortages in the supply of younger, competent women for such jobs. One of the important factors in this situation is that many mature women seeking employment have not been employed for some time and have lost skill and confidence. It was the objective of these programs of training to deal particularly with this factor.

These programs were conceived by the Women's Bureau and the Bureau of Employment Security of the U. S Department of Labor, and were developed in cooperation with a number of national women's organizations. The plan coming out of this cooperative effort was one for testing a program worked out by local women's organizations and local State employment service offices for re-training older women for employment by actual operation of pilot projects in local communities.

Although these programs were conceived at the national level, each test city developed its own methods and program to meet the needs in the local situation; not only did program and emphasis vary among the five test cities but the groups participating also varied. In Cleveland and Denver the Board of Education in cooperation with the Bureau of Employment Security developed programs for intensive typing and stenographic refresher training of women unable to meet required performance levels. The New York City program, carried on by a private welfare agency, was concerned with how best to help older women earn a living. The fourth program was that of a Milwaukee bank which developed ways to use older women as business machine operators on a part-time work-schedule in order to meet an acute labor shortage. These programs resulted in the successful employment of older women.

While the number of women affected in each of these programs was relatively few and the programs themselves exploratory and experimental in nature, this report, which provides detailed but economical descriptions of the ways in which these projects were developed, of policies established, and of the plan of organization and the methods of administration used in them, furnishes excellent patterns of successful action by public adult education programs and the employment service, by a private welfare agency, and by a business enterprise in creating and administering such a training program for older workers. These tested patterns should be useful to other communities planning projects with similar objectives.

Part II of this Bulletin provides a well-organized digest of statistics and other data which illuminate the employment problems of older women workers with special reference to office work.

This Bulletin is a valuable resource in providing a practical blueprint for action by community groups in meeting the employment problems of older workers. We say "older workers" advisedly since the issues involved and the techniques for dealing with them developed in these programs are equally applicable to problems of employment of older men as well as older women.

G. F. McCOY
Washington, D. C.

BOOKS RECEIVED

Books received since January 1 are acknowledged in the following list.

Das Alter als Schicksal und Erfüllung, Third Edition, by A. L. Vischer, Benno Schwabe & Co. Verlag, Basel, 1955, 259 pages, Fr. 11.50.

Education for Later Maturity, a handbook compiled by Wilma Donahue, William Morrow & Company, Inc., New York, 1955, 338 pages, $4.50 (reviewed in this issue, page 220).

Occupational Mobility in the U. S., 1930-1940, by A. J. Jaffe and R. O. Carleton, Columbia University Press, New York, 1954, 105 pages, paper bound, multilithed, $2.75.

JOURNAL OF GERONTOLOGY

VOLUME 10, Number 2

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Section C

Organization Section and Index to Current Periodical Literature

ORGANIZATION SECTION

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Biological Sciences	
James A. Hamilton (San Francisco)	1956
Henry S. Simms (New York)	1957
Clinical Medicine	
Joseph T. Freeman (Philadelphia)	1956
Arthur A. Hellbaum (Oklahoma City)	1957
Psychological and Social Sciences	
Wilma Donahue (Ann Arbor)	1956
James E. Birren (Bethesda)	1957
Social Work and Administration	
Julius Weil (Cleveland)	1956
Florence E. Vickery (San Francisco)	1957
General Membership Division	
Georgia F. McCoy (Washington, D. C.)	1955
Oscar J. Kaplan (San Diego)	1957

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Lawrence K. Frank	1947	Ernest W. Burgess	1952
Joseph C. Aub	1948	E. Vincent Cowdry	1953
Clive M. McCay	1949	Anton J. Carlson	1954

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Summary of the Minutes of the Ninth Annual Meeting of the Council and Members of the Corporation of the Gerontological Society, Inc.

The meeting was called to order at 1:00 p.m. on December 29, 1954, in the East Wing of the cafeteria, University of Florida, Gainesville, Florida. Members of the Council present were: A. J. Carlson, O. A. Randall, E. V. Cowdry, N. W. Shock, J. E. Kirk, A. I. Lansing, G. F. McCoy, W. Donahue, and J. A. Hamilton.

The treasurer presented the audited report for the year 1953 and a projected statement for the current calendar year and reported that the finances were in fair condition. This fairly satisfactory economic situation is due partly to a grant of $2500 from the Forest Park Foundation for this year and partly to an increase in the number of subscribers.

The Editor of the Journal of Gerontology reported that during the past year approximately 50 per cent of the papers were in the biologic and clinical areas and approximately 50 per cent in the psychologic and social science areas and that the Journal has a mailing list as of November 1 of 1756.

The budget for 1955 was presented by the treasurer and approved by the Council.

The secretary reported that the present membership is 1140 as compared with 950 in September 1953.

The Council acted upon the list of members recommended by the appropriate committees for fellowship status and elected those recommended to fellowship in the various sections of the Society. Notification will be sent by the Secretary of the Society to members elected to Fellow status.

A contribution of $50.00 was authorized to the National Society for Medical Research for 1955.

The Council appointed Dr. Wilma Donahue as the Society's representative on the council of the AAAS for three years.

A decision was reached to authorize the local committees of future annual meetings to accept and establish appropriate fees for commercial exhibits.

It was decided that membership certificates be made available on request to *Fellows* of the Gerontological Society at a fee to be set to cover the cost, the details to be worked out by the Membership Committee for the coming year. The Membership Committee is also charged with investigating the advisability of issuing membership cards to all members.

The Council recommends that the Program Committee for future meetings arrange for a general session to improve communication between the various sections of the Society and to coordinate their efforts without sacrifice of the opportunity for individual sections to develop individual technical programs.

Council approval was given for the appointment of committees to:

1. reevaluate and study the policy and content of the Newsletter,
2. review television programs of problems of aging being compiled, with authority to decide on possible sponsorship without financial cost to the Society,
3. develop state and local organizations and their affiliation with the Gerontological Society,
4. review the constitution and by-laws of the Gerontological Society,
5. explore the possibilities of obtaining research fellowships for young investigators in the field of gerontology and set up machinery for evaluation of the qualifications of the applicants and administration of fellowship funds,
6. study and recommend methods for bringing to the attention of young people of college level research problems within the field of gerontology with the purpose of promoting research.

N. W. Shock
Secretary

Summary of the Minutes of the Seventh Annual Meeting of the Gerontological Society, Inc.

The meeting was called to order by President Carlson at 2:00 p.m., December 30, 1954, in the Student Union Auditorium, University of Florida, Gainesville, Florida.

Dr. Carlson presented a report on actions and recommendations of the Council.

It was voted to hold the Eighth Annual Meeting of the Society in Baltimore, Mary-

land, during the latter part of October, since circumstances made it inadvisable to meet in Ann Arbor as voted in 1953. Arrangements have been completed to hold the Eighth Annual Meeting at the Sheraton Belvedere Hotel, Baltimore, Maryland, October 27, 28, 29, 1955. It was also voted to hold the Ninth Annual Meeting in Chicago sometime in October 1956.

All actions and recommendations of the Council were confirmed.

The following officers and members of the Council were elected:

President	Ollie A. Randall
President-elect	William B. Kountz
Secretary	Nathan W. Shock
Treasurer	John Esben Kirk

Council members:

Biological Sciences	*To Serve Through*
James A. Hamilton	1956
Henry S. Simms	1957
Clinical Medicine	*To Serve Through*
Joseph T. Freeman	1956
Arthur A. Hellbaum	1957
Psychological and Social Sciences	
Wilma Donahue	1956
James E. Birren	1957
Social Work and Administration	
Julius Weil	1956
Florence E. Vickery	1957
General Membership Division	
Georgia F. McCoy	1955
Oscar J. Kaplan	1957

The past-president, Dr. Anton J. Carlson, will also be a member of the Council for 1955.

The Society expressed its appreciation to the Committee on Local Arrangements and the University of Florida for their work and contributions toward the success of the meeting.

N. W. Shock
Secretary

INDEX TO CURRENT PERIODICAL LITERATURE

NATHAN W. SHOCK

Gerontology Section
National Heart Institute
National Institutes of Health

THE SUBJECT categories are those in *A Classified Bibliography of Gerontology* and *Geriatrics* by Nathan W. Shock published by Stanford University Press, Stanford, California (1951). Only major headings are used and the Roman numerals correspond to those given in the bibliography. In so far as possible, references are classified according to organ systems. Thus most of the material on Geriatrics will be found under the organ system involved in the disease. Cross references are indicated by number at the end of each section. When available, abstract references are given (*B.A.—Biological Abstracts; P.A.—Psychological Abstracts;* and *P.I.—Population Index*). Abbreviations for journals are those used in *A World List of Scientific Periodicals Published in the years 1900-1933*, 2nd Edition. For journals not listed, abbreviations were devised according to the general rules used in the above source. It is impossible to cover all journals and list all papers concerned with aging and the aged. Authors and publishers are requested to call attention to publications or to send reprints to the Gerontology Section, Baltimore City Hospitals, Baltimore 24, Maryland. The assistance of the Forest Park Foundation, Peoria, Illinois, in the preparation of these materials is gratefully acknowledged.

GERONTOLOGY

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BIOLOGY OF AGING

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IV. Longevity

(case reports, drugs, heredity, marriage, occupation, and sex differences)

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IV-C. Longevity: *Comparative Physiology*

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